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To Whom It May Concern:

I would like to clarify the study findings from our recent research into fecal protozoa and bacteria present in mussels along the central California coast. The purpose of the studies was to try out a new monitoring method for potentially pathogenic microbes, by outplanting sentinel mussel batches (in a manner similar to the State Mussel Watch Program that tests for pesticide and metal contaminants), and then testing them for selected fecal protozoa and bacteria. Mussels were outplanted and collected near sites exposed to livestock runoff, human sewage (outfalls or septic tanks), or at sites distant to both sources. Sites ranged from as far north as Bodega Bay to as far south as Morro Bay, CA. Mussels were collected during the wet and dry seasons over the course of 3 years as the project evolved.

Results from the bivalve bacteria study were presented at the Sea Otter Research Conference in Santa Cruz, CA in 2005, and have been submitted for publication. Bacteria of interest in this study included *Campylobacter* spp., *Salmonella* spp., *Escherichia coli*-O157, *Plesiomonas shigelloides*, *Vibrio* spp, and *Clostridium perfringens*. No *Campylobacter* spp., *Salmonella* spp., *Escherichia coli*-O157 were detected in mussels during the study, and all of the other potentially pathogenic bacteria were detected but are also known to have environmental reservoirs where they may persist indefinitely after introduction to aquatic environments, making the significance of detecting these bacteria in the sentinel mussels unclear with regard to fecal pathogen pollution and public health implications.

One of the study findings was that mussels collected close to human sewage sources (sewage outfalls or septic tanks) along the central California coast were 39 times as likely to have any of the study bacteria compared to the mussels collected from locations distant to these sources ($P=0.01$). Our study sample size does not have the statistical power to distinguish between sewage outfalls and septic tank exposure, and the finding of increased odds of detecting bacteria in mussels exposed to human sewage sources could be explained by either increased pathogen loading from these sources, or other environmental variables such as increased nutrient flux or temperature that could enhance bacterial growth.

With regard to the Morro Bay sewage outfall, the two sentinel mussel batches that were tested for bacteria had similar findings to the other mussel site outside Morro Bay (near Motel Point), and the sites inside Morro Bay. Mussels from the 2002 batches were negative for all bacteria except low levels of *Clostridium perfringens*, and mussels from the 2003 batches were negative

Item 6, Attachment 2
May 11-12, 2006 Meeting
Morro Bay/Cayucos WWTP

except for low levels of *Vibrio alginolyticus*. We did not find that the Morro Bay sewage outfall was a point source for fecal bacteria loading into the nearshore ecosystem in this study.

As for fecal protozoa, the occasional detection of *Cryptosporidium* spp. in mussels outplanted near the Morro Bay sewage outfall (Miller et al., 2005) during the 2001-2004 study was similar to the detection in mussels from the other mussel sites located outside and inside Morro Bay. There was no statistical association between *Cryptosporidium* detection in mussels collected from sites considered higher risk for human sewage exposure, livestock runoff, or lower risk sites for fecal pollution.

Our limited *Toxoplasma* bivalve work is unpublished but is discussed in the 2004 letter from Dr. Patricia Conrad to Mr. Bruce Keogh, regarding the *Toxoplasma*-negative results in four batches of 30 mussels tested quarterly in 2003-2004 that were outplanted on the Morro Bay sewage outfall buoy with the cooperation of the sewage treatment plant. We did not detect *Toxoplasma* RNA in those mussels, but the assay detection limitations are discussed in Dr. Conrad's letter. We are hopeful that with continued effort we will be able to improve our assays to detect the low levels of *Toxoplasma* that may still pose significant health risks to humans and animals in nearshore ecosystems. At present we are trying to find sufficient funds to support the development of critical reagents and validation of improved methods for the concentration and detection of *Toxoplasma gondii* oocysts. Please contact Dr. Melissa Miller, Dr. Patricia Conrad, or me for additional details if needed on these collaborative research projects.

Sincerely,

Woutrina Miller, DVM, MPVM, PhD

Miller, W.A., M.A. Miller, I.A. Gardner, E.R. Atwill, A.C. Melli, M. Harris, J. Ames, K. Worcester, N. Barnes, D. Jessup, and P.A. Conrad. 2005. New genotypes and factors associated with *Cryptosporidium* detection in mussels (*Mytilus* spp.) along the California coast. *International Journal for Parasitology* 35:1103-1113.