Monitoring and Mitigation to Address Fecal Pathogen Pollution along California Coast

Proposition 50 Coastal Management Program
California State Water Board Agreement No. 06-076-553

Applied Marine Sciences, Inc.
University of California Davis
California Department of Fish and Game
Marine Wildlife Veterinary Care and Research Center

Under the auspices of
Central Coast Long-term Environmental Assessment Program
Final Report

Monitoring and Mitigation to Address Fecal Pathogen Pollution along California Coast

Proposition 50 Coastal Management Program

Agreement No. 06-076-553

May 31, 2011

Submitted to:
California Regional Water Quality Control Board
Region 3
895 Aerovista Place, Suite 101
San Luis Obispo, CA 93401

Submitted by:
Applied Marine Sciences, Inc.
University of California Davis
California Department of Fish and Game
Marine Wildlife Veterinary Care and Research Center

Under the auspices of
Central Coast Long-term Environmental Assessment Program
Table of Contents

1. Executive Summary..................................................................................1-1
1.1. Report Organization..............................................................................1-1
1.2. Results for each Question...................................................................1-2

2. Background............................................................................................2-1
2.1. CCLEAN Program Description..............................................................2-1
   2.1.1. Objectives.....................................................................................2-1
2.2. Proposition 50 Grant Program..............................................................2-2
   2.2.1. History..........................................................................................2-2
   2.2.2. Focus.............................................................................................2-2
   2.2.3. Application Process.......................................................................2-2

3. Project Description..................................................................................3-1
3.1. Team.................................................................................................3-1
3.2. Goals and Objectives..........................................................................3-2
3.3. Pathogen Indicators and Fecal Pathogens Studied...............................3-3
3.4. Questions to be answered...................................................................3-4
3.5. Project Scope.......................................................................................3-5
   3.5.1. Field Methods...............................................................................3-5
   3.5.2. Analytical Methods.......................................................................3-14

4. Results of each Question.........................................................................4-1
4.1. What are the spatial and temporal patterns in pathogen indicator concentrations and what is the relationship between pathogen indicators and actual fecal pathogens in: Influent and Effluent, Rivers and Streams, Ocean Water and Mussel Tissue, Storm Runoff, and Wetlands? ...............................................................4-1
   4.1.1. Wastewater Influent and Effluent.......................................................4-1
   4.1.2. Rivers and Streams........................................................................4-14
   4.1.3. Ocean and Mussels.........................................................................4-32
   4.1.4. Stormwater.....................................................................................4-34
   4.1.5. Wetlands.........................................................................................4-37
4.2. Loading Estimates................................................................................4-40
4.3. What is the relationship between exceedences of water quality objectives for bacteria and actual concentrations of known fecal pathogens?.................................................................4-46
4.4 Are mussels better indicators of ocean bacterial water quality than water samples? ..........................................................4-49
4.5. Which of the three microbial source tracking methods evaluated appears most promising as a tool for environmental surveillance, and what can we learn about trends in human versus animal sources of fecal pollution along the central coast of California? .................................................................4-50
4.6. Is there a connection between fecal pathogens from terrestrial sources and those found in sea otters? ..........................................................................................................................4-58
4.7. Are wetlands effective for reducing fecal pathogen loads in polluted water and what wetland characteristics are most critical for pathogen reduction?...............................................................4-76

5. Conclusions............................................................................................5-1

6. Acknowledgements..................................................................................6-1

7. References...............................................................................................7-1

8. APPENDIX A..........................................................................................8-1
TABLE INDEX

Table 1. Project team .................................................................................................................. 3-1

Table 2. Monitoring and Mitigation to Address Fecal Pathogen Pollution along California Coast personnel responsibilities ..................................................................................................... 3-1

Table 3. Overview of sample types and collection techniques ................................................. 3-5

Table 4. Sample types, test parameters, sampling frequency and applicable water-quality stressors ................................................................................................................................ 3-6

Table 5. Locations of stream water monitoring sites for fecal pathogen project ....................... 3-9

Table 6. Site names and coordinates for paired mussel and nearshore water sampling locations for P3 Project ................................................................. 3-10

Table 7. Locations of stormwater monitoring sites for P3 Project ........................................... 3-11

Table 8: Experimental conditions used in the pathogen tank exposure experiments. ............... 3-12

Table 9. Volumes and dilutions for membrane filtration process and bacterial culture in river (R) and seawater (S) samples ................................................................................................... 3-15

Table 10. Primers used for qPCR assays. .................................................................................. 3-16

Table 11. Sample dates, type and flow on given sample date as well as weather information close to sample date ..................................................................................................................... 4-2

Table 12. Percent prevalence of each organism in all effluent and influent samples for each of the 4 treatment plants .................................................................................................................. 4-3

Table 13. Results from analysis of variance for differences among sites in the recovery of spiked protozoal oocysts. .................................................................................................................. 4-3

Table 14. Influent and effluent loads of selected fecal pathogens (microbe concentration/ 100 ml or 10 L) for 4 wastewater treatment facilities in the Monterey Bay region of central California. .................................................................................................................. 4-4

Table 15. Influent and effluent loads of selected fecal pathogens (microbe concentration/ 100 ml or 10 L) for 4 wastewater treatment facilities in the Monterey Bay region of central California: Measures of central tendency using ROS methods. ........................................... 4-7

Table 16. Temporal and spatial differences among sites for concentrations of indicator bacteria and fecal pathogens at four wastewater treatment facilities in central California .......... 4-12

Table 17. Results of stepwise multiple regressions to determine relationships between indicator bacteria or fecal pathogens in influent and local rainfall .................................................. 4-12
Table 18. Percent removal of indicator bacteria and fecal pathogens from wastewater during treatment in four wastewater treatment facilities in the Monterey Bay area. .................... 4-13

Table 19. Prevalence of bacterial and protozoal pathogens in monthly surface water samples by site in the Monterey Bay region of California (2007-2008). .......................................................... 4-17

Table 20. Simple logistic regression analysis of variables with significant association of pathogenic bacteria detection in surface water samples from April, 2007 to September, 2008.................................................................................................................................... 4-20

Table 21. Multivariate logistic regression analysis of risk factors significantly associated with *Vibrio* spp. detection in surface water samples form April, 2007 to September, 2008. .... 4-21

Table 22. Significant Spearman correlations between *Bacteroidales* markers, indicator bacteria, and specific pathogen detection for all sites combined, either with non-detects (test-negative samples) included as zero values (top) or omitted (bottom). ......................................................... 4-22

Table 23. Summary statistics for pathogen concentrations in rivers using regression on order statistics (ROS). ................................................................................................................. 4-30

Table 24. Pathogen prevalence in ocean water and mussels in central California. ............. 4-32

Table 25. Summary statistics for pathogen concentrations in ocean samples using regression on order statistics (ROS). ................................................................................................................. 4-33

Table 26. Summary table of stormwater pathogen concentration means and ranges for each site. ............................................................................................................................................ 4-34

Table 27. ANOVA results for stormwater data with significant differences by pathogen. *A Posteriori* test used was Tukey HSD (Honestly Significant Difference) test. .................. 4-36

Table 28. Summary of central tendency using regression on order statistics (ROS) for stormwater samples. ................................................................................................................................................. 4-36

Table 29. Prevalence of *Cryptosporidium parvum* and *Giardia lamblia* in the Tembladero Slough Constructed Wetland. ............................................................... 4-37

Table 30. Total coliform and *Escherichia coli* detection in water samples collected from source water and the Tembladero Slough Constructed Wetland using the Colilert-18 test. .... 4-38

Table 31. Total coliform, fecal coliform, and enterococci detection in water samples collected from source water and the Tembladero Slough Constructed Wetland using membrane filtration. .......................................................................................................................... 4-38

Table 32. Wilcoxon Score (U) of seasonality and rainfall events with protozoa in Tembladero Slough Constructed Wetland. ............................................................................................ 4-39

Table 33. Spearman’s rho correlation of water quality parameters with protozoa in Tembladero Slough Constructed Wetland. ............................................................................................ 4-39
Table 34. Percent recovery of spiked protozoal oocysts in subsamples of coastal surface water from the Monterey Bay area. .........................................................................................................................4-42

Table 35. ANOVA results to determine significant differences in concentrations of indicator bacteria and fecal pathogen concentrations among discharge sources using transformed data \[\log (x+1)\]..........................................................................................................................4-45

Table 36. ANOVA results to determine significant differences in daily loads of indicator bacteria and fecal pathogens among discharge sources using transformed data \[\log (x+1)\]. ........4-46

Table 37. Proportion of fecal indicator bacterial counts exceeding standard water quality criteria in surface water samples collected along the central coast of California (2008-2010). ....4-47

Table 38. Univariate analysis of associations between fecal indicator bacterial counts exceeding standard water quality criteria for recreational contact and enteric pathogen detection in surface water samples collected along the central California coast (2008-2010)..........4-48

Table 39. Multivariate model of associations between fecal indicator bacterial counts exceeding standard water quality criteria for recreational contact and enteric pathogen detection in surface water samples collected along the central California coast (2008-2010).........4-48

Table 40. Site characteristics with respect to land use, main upstream fecal source, and Bacteroidales marker detection in surface waters. ........................................................................................................4-50

Table 41. Conditional probability, sensitivity, specificity, positive and negative predictive value, and prevailing rate for four 16S rRNA-based qPCR assays targeting universal, human-, cow-, and dog-specific Bacteroidales markers in surface waters........................................4-51

Table 42. Simple logistic regression of total coliform: fecal coliform ratios (cfu/100ml) associated with detection of enteric bacterial pathogens in river and ocean water samples from central California (2008-2010). ..................................................................................................................4-56

Table 43. Associations between total coliform: fecal coliform ratios (cfu/100ml) and enteric bacterial pathogen detection for river and ocean water samples with total coliform counts >1000 (cfu/100ml). ..................................................................................................................4-56

Table 44. Associations between total coliform: fecal coliform ratios (cfu/100ml) and specific bacterial pathogen detection for river and ocean water samples with total coliform counts >5000 (cfu/100ml). ..................................................................................................................4-57

Table 45. Associations between total coliform: fecal coliform ratios (cfu/100ml) and specific bacterial pathogen detection in stormwater samples from the central California coast (Simple logistic regression). .................................................................4-57

Table 46. Prevalence with 95% binomial confidence intervals (CI) for bacterial pathogens cultured from terrestrial and marine animal species in the Monterey Bay region of California, 2007-2010. ........................................................................................................4-60
Table 47. Univariate logistic regression of risk factors associated with detection of bacterial pathogens cultured from terrestrial and marine animal species in the Monterey Bay region of California, 2007-2010. ................................. 4-62

Table 48. Multivariate logistic regression of risk factors associated with detection of bacterial pathogens cultured from terrestrial and marine animal species in the Monterey Bay region of California, 2007-2010. ................................. 4-64

Table 49. Antimicrobial resistance patterns of *Salmonella*, *Vibrio cholerae*, and *Vibrio parahaemolyticus* isolated from wastewater, river, and sea otter fecal samples. ................ 4-67

Table 50. Prevalence with 95% binomial confidence intervals (CI) of protozoal pathogens recovered from terrestrial and marine animal species from areas in or near Monterey Bay, California during 2007-2010. ......................................................... 4-68

Table 51. Univariate logistic regression of variables with detection of protozoal pathogens recovered from terrestrial and marine animal species from areas in or near Monterey Bay, California during 2007-2010. ......................................................... 4-69

Table 52. Multivariate logistic regression of variables with detection of protozoal pathogens recovered from terrestrial and marine animal species from areas in or near Monterey Bay, California during 2007-2010. ......................................................... 4-71

Table 53: Effect of water flow rate and salinity on *Cryptosporidium* oocyst and *Giardia* cyst counts in non-vegetated mesocosm tanks (Univariate analysis). .......................... 4-77

Table 54. Effect of defined vegetation parameters on counts of *Cryptosporidium* and *Giardia* in mesocosm tanks (univariate analysis). ......................................................... 4-78

Table 55. *Cryptosporidium* oocyst detection in relation to sample time and vegetation presence, type and configuration in mesocosm tanks (multivariate estimates). ................................. 4-80

Table 56. *Giardia* cyst detection in relation to flow rate, sample time and vegetation presence, type and configuration in mesocosm tanks (multivariate estimates). ................................. 4-82

Table 57. Site-specific detection of *C. parvum* and *G. lamblia* in water from Tembladero Slough Constructed Wetland and source water. ......................................................... 4-82

Table 58. Detection of Total Coliforms and *E. coli* in water from Tembladero Slough Constructed Wetland and source water using Colilert-18 testing. ................................. 4-83

Table 59. Detection of total coliforms, fecal coliforms, and Enterococci in Tembladero Slough Constructed Wetland and source water using membrane filtration. ................................. 4-83

Table 60. Associations between seasonality, rainfall events and *Cryptosporidium* or *Giardia* counts in the Tembladero Slough Constructed Wetland (Wilcoxon Score U) .................. 4-84

Table 61. Associations between water quality parameters and *Cryptosporidium* or *Giardia* counts in the Tembladero Slough Constructed Wetland (Spearman’s rho correlation) .................. 4-84
FIGURE INDEX

Figure 1. Location of sampling sites for wastewater, surface water, ocean water, mussels, and constructed wetland ............................................................................................................. 3-7

Figure 2: Comparison of California bulrush configuration. a: Buffer configuration, b: Channel configuration...................................................................................................................... 3-13

Figure 3: Map of the Tembladero Slough Constructed Wetland Sampling Sites................................................. 3-14

Figure 4 (a, b). Mean recoveries of spiked protozoal oocysts into influent and effluent samples (n = 3) from four wastewater treatment facilities..................................................................... 4-8

Figure 5 (a, b). Mean densities of indicator bacteria and fecal pathogens in samples collected from four wastewater treatment facilities in the Monterey Bay area............................................. 4-10

Figure 6 (a, b). Mean densities of indicator bacteria and fecal pathogens collected approximately quarterly in 2007 and 2008 from four wastewater treatment facilities in the Monterey Bay area..................................................................................................................................... 4-11

Figure 7. Mean indicator bacteria concentrations at 10 rivers along the central California coast from April, 2007 to September, 2008........................................................................................................ 4-14

Figure 8. Mean monthly indicator bacterial mean concentrations at 10 rivers along the central California coast from April, 2007 to September, 2008.............................................................................. 4-15

Figure 9. Box plots of (top) site-specific variation of universal Bacteroidales concentration from North to South, and (bottom) seasonal variation for universal Bacteroidales (light grey) and fecal coliforms (dark)......................................................................................................... 4-16

Figure 10. Mean Vibrio concentrations at 10 rivers along the central California coast from April, 2007 to September, 2008. .......................................................................................................... 4-18

Figure 11. Mean monthly Vibrio concentrations at 10 rivers along the central California coast from April, 2007 to September, 2008. .......................................................................................................... 4-18

Figure 12. Threshold concentration ranges for universal Bacteroidales (top) and total coliforms (bottom), with resulting PQ values greater than 66% for data from all sites combined and separated by average site salinity (freshwater salinity: <0.5 ppt). ................................................................. 4-24

Figure 13. Threshold concentration ranges for fecal coliforms (top) and enterococci (bottom), with resulting PQ values greater than 66% for all sites combined, and separated by average salinity (freshwater salinity: <0.5 ppt). .................................................................................................................. 4-25

Figure 14. Stormwater load estimates for the Pacific Grove site at Greenwood Park........................................... 4-41
Figure 15. Stormwater load estimates for the Santa Cruz site at Woodrow Ave. ..................... 4-41

Figure 16. Mean concentrations of indicator bacteria and fecal pathogens from wastewater effluent, surface waters (streams and rivers) and storm runoff. .......................... 4-44

Figure 17. Mean daily loads (concentration x flow) of indicator bacteria and fecal pathogens from wastewater effluent, surface waters (streams and rivers) and storm runoff........... 4-45

Figure 18. *Salmonella* Typhimurium dendrogram showing intra-species relatedness of Pulsed Field Gel Electrophoresis (PFGE) fingerprints after *XbaI* digestion.......................... 4-65

Figure 21. Effect of vegetation type and configuration on *Giardia* cyst detection per 50 mL water in relation to the presence or absence of vegetation in mesocosm tank studies. ............ 4-79

Figure 22. Effect of vegetation presence (California bulrush in buffer configuration-Dashed line) or absence (Solid line) on *Giardia* cyst detection in 50 mL water sub-samples from mesocosm tanks. ........................................................................................................ 4-81
1. Executive Summary

1.1. Report Organization

Coastal waters worldwide have been significantly influenced by human activities, as they are adjacent to densely populated areas and support a wide range of transport, commercial and recreational uses. Urbanization-associated impairments of nearshore water quality can result from enrichment of nearshore marine waters by nutrients and chemical and biological pollutants that are transported from terrestrial watersheds to the ocean in ever-increasing quantities. Even after reaching the ocean this pollutant load poses health risks to humans and animals, and the degree of risk from marine dispersal of anthropogenic chemicals and pathogens may be greatly under-estimated. Fecal indicator bacteria (FIB) that normally reside in the gastrointestinal tract of humans and animals are used throughout the world to assess the microbiological quality of drinking and recreational waters. In the United States, FIB are used to define bacterial water quality standards aimed at reducing health risks in waters used for recreation and aquaculture. Groups of standard FIB monitored in water include total and fecal coliforms, *Escherichia coli*, and enterococci, and are considered as indicators of health risk in epidemiologic and quantitative microbial risk assessment (QMRA) studies.

To date, many monitoring programs have focused only on FIB measurements and do not test for the presence or absence of known pathogens, partly due to associated costs and expertise required for pathogen testing. However, substantial evidence has been collected challenging the usefulness of FIB data as a predictor of actual disease risk from contact with polluted water. A few limitations of using standard FIB to represent pathogens in water include the fact that FIB have the ability to multiply in the environment, they are not host-specific, and absence of FIB does not necessarily mean that pathogens are also absent. Consequently, alternative indicators of fecal pollution are needed that address the weaknesses of standard FIB for protection of human health. Ideally, these indicators would decay at similar rates as pathogens, be present at high concentrations in fecal sources, and be present at low concentrations in unpolluted environments. An added benefit of using alternative indicators is that in some cases host sources of fecal contamination can be identified.

The goals of this research program were to use both laboratory and field approaches to investigate issues related to water quality monitoring and mitigation of fecal pathogen pollution along the central California coast. Our specific objectives were to 1) evaluate water quality monitoring approaches by characterizing the relationships between FIB and enteric pathogen detection in a broad range of freshwater and marine surfacewaters along the central coast, 2) consider the relative importance of fecal pathogen loading from different sources, 3) evaluate whether filter-feeding estuarine or marine invertebrates (mussels) may be better indicators of water quality than direct water testing, 4) evaluate microbial source tracking techniques to distinguish between human and animal sources of fecal pollution, 5) characterize patterns of fecal pathogen shedding among terrestrial and marine animals, and 6) evaluate wetlands as a possible Best Management Practice (BMP) to mitigate impairments and improve surface water quality with respect to fecal pathogen pollution. The results are organized according to a series of priority questions that relate to the study goals and objectives, and are summarized in the following section, with additional details provided throughout the report.
1.2. Results for each Question

• What are the spatial and temporal patterns in fecal indicator bacteria and pathogens along the central California coast, and what is the relationship between fecal indicator bacterial concentrations and fecal pathogen detection in: wastewater influent and effluent; rivers and streams; ocean and mussels; stormwater; and wetlands?

Fecal indicator bacteria (total coliform, fecal coliform, Enterococcus, and Bacteroidales counts) were compared with direct detection of target bacteria (Campylobacter spp., Salmonella spp., Escherichia coli O157, and Vibrio spp.) and protozoa (Giardia and Cryptosporidium) in wastewater and surface water samples during a two-year period. Wastewater influent and effluent samples were tested quarterly from four wastewater treatment facilities. These facilities varied in the volume of wastewater handled per day, as well as the technologies utilized for wastewater processing. A significant reduction of pathogens between pre- and post-treatment was noted for all 4 facilities, but with differences among facilities in removal efficiency of FIB and enteric pathogens.

Water samples from ten coastal river sites were tested monthly over two years. FIB and enteric pathogen detection was both common and widely distributed between the ten coastal rivers, although detection was not highly correlated with sampling during the wet or dry seasons. Stormwater was sampled from three sites, and ocean water and mussels were sampled quarterly from six sites. FIB and specific pathogen detection was less common in these nearshore marine samples, when compared to river or stormwater. Pathogen trends in stormwater were similar to other sample types, with the protozoa Cryptosporidium and Giardia detected most often, followed by Salmonella and Vibrio spp., and little or no Campylobacter or E. coli-O157:H7 detection.

Quarterly testing of water collected from multiple sites in the Tembladero Slough constructed wetland showed that pathogens were detected most often in the slough sourcewater and at the inflow site, and less frequently as water moved down through the wetland. The ability to predict pathogen occurrence in relation to FIB threshold levels was evaluated using a weighted PQ measure that showed the universal Bacteroidales genetic marker had a comparable or greater mean predictive potential than standard FIB. We found that measures of traditional indicator bacteria, including coliforms and enterococci, correlated detection of some, but not all bacterial and protozoal pathogens in this study. Collectively our study findings suggest that monitoring for indicator bacteria alone may not provide sufficient information to minimize public contact with fecal pathogens in surface waters. We recommend utilizing a combination of FIB and specific pathogen assays to provide the most useful and accurate perspective regarding the presence, relative abundance, and contributing sources of fecal contamination in environmental water samples.

• How can fecal pathogen loading of nearshore ecosystems be compared across the full range of surface water inputs to the ocean?

This question emerged during the course of the study as a key synthesis question that could help resource managers more accurately assess local risks from water contact, and prioritize management strategies to minimize coastal pollution. We used data collected from different sources (i.e., streams and rivers, storm runoff and wastewater), combined with data on flow rates
and the number of potential loading sources by area, to make preliminary estimates of relative loading and to identify gaps where future study and data compilation is needed to improve the risk estimates. In some cases, fecal pathogen inputs were identified from all three sources (streams and rivers, storm runoff and wastewater) and were not dominated by any single source. Given the inherent uncertainty in our load estimates and considering only the days on which sampling occurred, the average daily ocean input of *Vibrio cholerae* was significantly greater for streams and rivers, while wastewater was the major contributor for *Giardia*. In contrast, *Cryptosporidium* and *V. parahaemolyticus* inputs were not significantly different among sources. Collectively our data suggest that discharge of pathogens in water originating from streams, rivers and storm runoff poses a greater risk to human health than offshore, deep-water discharges of wastewater effluent along the central California coast due to the absence of water treatment, limited pathogen dilution, and direct shoreline discharge patterns associated with the inland surface water sources.

• *What is the relationship between exceedences of water quality objectives for fecal indicator bacteria (FIB) and fecal pathogen detection in surface water samples?*

    California has set cutoffs for FIB counts to ensure public safety during water contact recreation and consumption of shellfish harvested from surface water bodies. In the current study, stormwater samples most commonly exceeded water quality (FIB) criteria, followed by river/stream/slough samples, and finally ocean water. Associations between FIB exceedances and specific pathogen detection varied by water sample type and pathogen group. Of all target pathogens, only *Cryptosporidium* detection was significantly associated with total coliform levels that exceeded current water quality criteria cutoffs. High fecal coliform counts were more closely associated with the presence of specific pathogens in surface water: *Cryptosporidium*, *Giardia*, *Salmonella*, and *V. parahaemolyticus* detection were all significantly associated with fecal coliform exceedences, while high enterococcal counts were predictive of *Giardia* and *V. parahaemolyticus* detection in surface water. These findings generally support the continued use of water quality criteria using FIB cutoffs for predicting health risks during recreational water contact and shellfish harvest. However, the lack of association between presence of some pathogens and FIB exceedences supports the concept that “absence of evidence is not necessarily evidence of absence”, meaning that enteric pathogens may still be present in surface waters with acceptable FIB levels, as was observed in the current study. This finding underscores the need to consider using multiple, or alternative water quality monitoring practices to improve our ability to predict pathogen presence and minimize health risks associated with water contact. Quantitative Microbial Risk Assessment is one framework that can be used to more comprehensively consider, characterize, and predict health risks associated with different beneficial uses.

• *Are mussels better indicators of ocean microbial water quality than seawater?*

    In the current study we compared time- and location-matched mussel sampling with collection and processing of 20 L volumes of seawater. Shellfish, including mussels, clams and oysters have all been suggested as more sensitive bioindicators of water quality in aquatic ecosystems, when compared to water “grab” samples. However, using whole mussel homogenates we found no significant difference in pathogen detection between time- and location-matched mussels and seawater. For example, the prevalence of *Giardia* and *Vibrio* species detection differed by less than 2%. However, some notable differences were observed:
Campylobacter and Salmonella were detected in seawater when mussels tested negative, with 10% and 5% pathogen prevalence, respectively, in seawater. Similarly, Cryptosporidium oocysts were detected in 26% of seawater samples, but only 6% of mussel batches. Based on the weather and water quality characteristics present during our sampling efforts, we suggest that bivalves may be most useful as bioindicators when sampled during “high-risk” periods for fecal contamination of aquatic ecosystems, such as during or after storm events.

• Which of three microbial source tracking methods is most promising and what can be learned about trends in human versus animal sources of fecal pollution?

Evaluating microbial source tracking (MST) techniques for distinguishing human from animal sources of fecal pollution along the central California coast was deemed important because the approaches for remediating human as compared to animal sources of fecal contamination are different, and because new molecular approaches are providing insights on source tracking that were previously unavailable with traditional phenotypic characterization methods. The three MST methods evaluated were 1) Bacteroidales assays, 2) an Enterococcus surface protein (esp) gene assay, and 3) total to fecal coliform ratios in water samples. The comparative study showed that the Enterococcus esp assay and total: fecal coliform ratios did not perform as well and do not show as much promise as Bacteroidales for future MST work. Based on the comparative MST results, Bacteroidales host-specific qPCR was then used to quantify fecal bacteria in water and provide insights into contributing host fecal sources. More than 140 surface water samples from 10 major rivers and estuaries within the Monterey Bay region were tested during 14 months with four Bacteroidales-specific assays (universal, human, dog, and cow). Bayesian conditional probability analysis was used to characterize the performance of Bacteroidales assays, and the ratios of concentrations determined using host-specific to universal assays indicated that fecal contamination from human sources was more common than livestock or dog sources in the coastal study sites.

• What are the patterns and risk factors for fecal pathogen shedding from central coast animals, and are the same types of fecal pathogens detected in sea otters as are detected in other marine and terrestrial animals?

Feces from domestic and wild animals were tested to determine the prevalence and genotypes of selected pathogens in the Monterey Bay region. Of 808 fecal samples tested between 2007 and 2010, 28% were positive for one or more target pathogens, and many of the same species detected in terrestrial animals were also isolated from sea otters. Giardia spp. were isolated most frequently, with an overall animal prevalence of 15%, followed by Campylobacter spp. (11%), Vibrio cholerae (9%), Cryptosporidium spp. (6%), Salmonella spp. (6%), and Vibrio parahaemolyticus (5%). Molecular characterization of Giardia and Cryptosporidium revealed both zoonotic and host-specific genotypes. Fifteen different Salmonella serotypes were detected, 11 of which were isolated from opossums, a non-native species introduced to coastal California. Risk factors associated with pathogen detection in animal feces included animal group, age class, gender, live-dead status, and season. These study findings provide insights that may be used to help prioritize animal management and water quality monitoring strategies.

• Are wetlands effective in reducing fecal pathogen loads in surface water, and, if so, what wetland characteristics are most important to achieve pathogen reduction?
Wetlands evaluation involved both controlled laboratory trials and field experiments. First, laboratory mesocosm tank models that simulated coastal wetlands were used to study specific variables believed to reduce the load of fecal pathogens present in contaminated runoff as it flows through a wetland. By introducing known quantities of specific pathogens at the inflow, and collecting samples under varying climatic and wetland restoration conditions (e.g., wetland length, vegetation configuration, salinity, flow rate), we determined the effects of these variables on reduction of pathogen concentrations in water traveling through the model wetlands. These studies revealed that the presence of vegetation enhanced removal of oocysts from fecally-polluted water at both fast and slow flow rates. The important role of vegetation in removal of waterborne protozoa should be considered in wetland reconstruction and management efforts for coastal ecosystems. Similar water measurements were conducted during quarterly testing at a reconstructed wetland at Temblader Slough, providing a larger scale, “real world” model of the ability of coastal wetlands to reduce fecal pathogen loads in surface waters. These larger-scale findings indicate that both the distance from various point source(s) of contamination and periodic rainfall events influence the efficiency of pathogen reduction in natural systems.

Considered collectively, our study findings provide important new insights for water quality managers working at all levels and in multiple disciplines. These include specific suggestions for improving water quality monitoring and mitigation efforts in order to optimize the balance between coastal development and safety of coastal marine waters for recreation, shellfish harvest and other beneficial uses such as threatened and endangered species protection. Publications and outreach materials related to this project will be posted on the website www.pathogenpollution.org as they become available.