Center for Water Resources
Annual Technical Report
FY 2004

Introduction

The UC Center for Water Resources is a multicampus research unit and a special program within the University of Californias Division of Agriculture and Natural Resources. The major function is to support research and extension activities that will contribute to the efficient management of water resources within the state. Meeting the needs of the urban, agricultural and wildlife sections from both water quality and quantity considerations is a goal of the Center. The Center has linkages to faculty on all UC campuses in the UC system and to extension personnel in each of the 58 counties. The Center can be reached by email at cwres@ucr.edu and our web site can be accessed at http://waterreseources.ucr.edu.

Research Program

The Water Resources Center funded 11 new projects and continued 7 projects for a total of $556,620.00 with nearly every UC Campus participating.

Dynamics of Point and Non-Point Source Fecal Pollution from an Urban Watershed in Southern California

Basic Information

Title:	Dynamics of Point and Non-Point Source Fecal Pollution from an Urban Watershed in Southern California
Project Number:	2003CA50G
Start Date:	9/1/2003
End Date:	8/31/2006
Funding Source:	104G
Congressional District:	48
Research Category:	None
Focus Category:	Ecology, Surface Water, Water Quality
Descriptors:	None
Principal Investigators:	Stanley B. Grant, Patricia Ann Holden, Brett franklin Sanders

Publication

13. Title: *The Dynamics of Point and Non-Point Source Fecal Pollution from an Urban Watershed in Southern California*

14. Statement of Critical Regional or State Water Problem

One of the most important environmental concepts to emerge in the past decade is the idea that non-point source pollution must be solved at the watershed-scale. EPA's total maximum daily load (TMDL) process is a formal approach for fostering watershed-scale management that requires[1]: (1) Establishing in-stream numeric targets for pollutants of concern. (2) Performing an inventory of all pollutant sources and their respective contribution to the impairment. (3) Identifying target pollutant levels that will yield the desired water quality goal, after factoring in seasonal variation, critical conditions, and a margin of safety. (4) Allocating pollutant loads to all point and non-point sources in the watershed. Relative to FIB and pathogen impairment, items (2) through (4) can present significant challenges, particularly in cases where the bulk of the contamination is from non-point sources such as urban or agricultural runoff, and/or distributed re-growth. One increasingly popular approach for addressing items (2) through (4) involves utilizing GIS-based modeling software that simulates pollutant loading into a river from point and non-point sources; for example, the EPA model known as Better Assessment Science Integrating Point and Non-point Sources (BASINS). To implement these software packages, the user is required to provide information about suspected or known sources of pollutants (e.g., from failing septic tank systems in the case of FIB). Pollutant inputs from runoff are handled by specifying build-up rates and maximum storage values of the contaminant by land-use type, together with geographically referenced land-use and hydrology data for the watershed of interest (e.g., see http://www.epa.gov/waterscience/ftp/basins3).

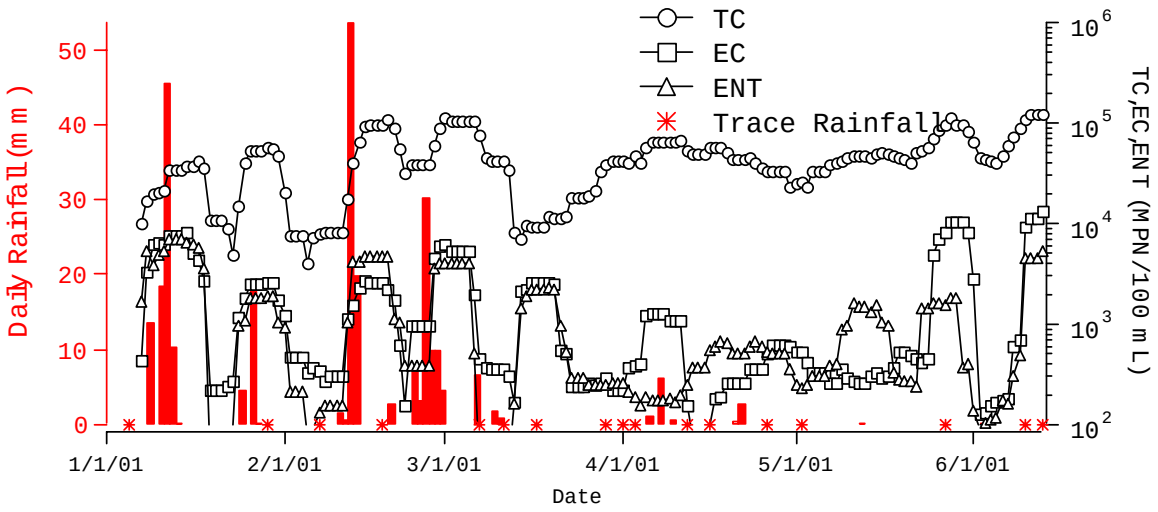

Figure 1. Daily FIB concentrations measured in the Fountain Valley Channel, which drains a coastal urban community in the Santa Ana River Watershed. Water from this stream was collected every day for six months beginning January 1, 2001. Samples were analyzed for Total Coliform (TC), *E. coli* (EC), and *Enteroccocus* (ENT) using defined substrate tests known commercially as Colilert and Enterolert, implemented in a 97 well quantitray format. FIB data were low-passed filtered (cut-off frequency of 1/week) to reduce high frequency noise.

The procedure described above conceptualizes FIB as static pollutants that accumulate, wash-off,

[1] USEPA (2000) Revisions to the Water Quality Planning and Management Regulation and Revisions to theNational Pollutant Discharge Elimination System Program in Support of Revisions to the Water Quality Planning and Management Regulation: Final Rule. Fed. Reg. 65:43586-43670.

(and perhaps die-off) at well-defined rates, but the reality is considerably more complex. As an illustration of this complexity, we present FIB measurements in a small (Q=0.025 m^3/s during dry-weather periods and Q=1 m^3/s) stream that drains roughly 600 ha of residential communities in Fountain Valley and Huntington Beach, on the south (coastal) edge of the SAR watershed (Fig. 1). The concentrations of FIB in urban runoff from this sub-drainage are strongly modulated by rainfall events that occurred during the storm season from January through April (note that FIB concentrations are plotted on a logarithmic scale, see right axis). Later in the season, the three indicator bacteria appear to respond to *trace* rainfall events during which there was no observable increase in stream discharge. Several features of these data warrant discussion:

- The peak TC concentrations appear to increase with each successive storm during the January to April time frame. Either FIB build-up very quickly over time (complete recovery in less than two weeks) or, more likely, there is a problem with the simple build-up/wash-off paradigm typically employed to model FIB input from non-point sources.
- After the storm season, TC concentrations in the channel continue to climb, reaching their highest levels (>10^5 MPN/100 mL) during the early summer when only trace rainfall was recorded. The fact that TC concentrations steadily climb during this period reflects some (non-obvious) unsteady process; e.g., perhaps a shift in the origin of the runoff toward residential irrigation runoff, or re-growth of TC in the sediments and/or water column.
- Ratios of the different FIB groups continuously change during the six-month study, reflecting a continuous evolution in the ecology and/or sourcing of these organisms. For example, the TC/EC ratios range from approximately 10 at the beginning of the study to >100 by the beginning of summer. The concentration of the EC and ENT groups are nearly identical during the stormy season, but then deviate significantly later, with ENT>>EC in some cases (e.g., the trace rainfall event in the middle of May), and ENT<<EC in other cases (e.g., the rainfall event in early April, and the trace rainfall event in late May).
- Some of the largest concentrations of EC and ENT coincide with trace rainfall events during the spring to summer period. This phenomenon does not appear to be related to wash-off per se, because many of the trace rainfall events were not accompanied by measurable increases in stream flow (data not shown). One possible explanation is that, by wetting the pavement, trace rainfall stimulates FIB blooms in the water column.

In summary, the most prominent features of the FIB data plotted in Figure 1 cannot be explained by a straight-forward build-up/run-off conceptualization of FIB shedding from urban watersheds and hence a deeper understanding of the underlying processes is required

15. Statement of Results or Benefits.

The field measurements and modeling studies proposed here are intended to provide greater insight into the factors that control the spatial and temporal distribution of FIB in urban watersheds, particularly the ephemeral urbanized systems found in southern California.

Specifically, this project will generate:

- Information on the sediment and water column ecology of FIB (specifically speciation and microbial diversity) in an urban watershed under both dry and wet weather conditions.
- Information on the temporal variability of in-stream FIB concentrations and loading, and the

effect of both local and external forcing.

- A mathematical model for predicting FIB concentrations (and loads) in urban streams that captures the dominant ecological and transport phenomena identified during the field phase of the project; this model will be fully tested against measured FIB levels at several sites, and over a variety of time-scales, ranging from hours to decades.

Insights obtained from the proposed project will assist watershed managers and planners in their efforts to identify and mitigate non-point sources of FIB pollution, as evidenced by the supporting letter and matching funds provided for this project by the Counties of Orange, San Bernadino, and Riverside. By attempting to incorporate these insights into a runoff model, we will create a new generation of transport/ecology modeling tools that engineers can use for designing and implementing watershed FIB and pathogen TMDLs in southern California and elsewhere. The proposed study is also interesting scientifically, because it will be among the first to investigate how storm flows affect the ecology of microbial populations (in this case FIB) in ephemeral watersheds. Additional benefits of the proposed project are accrued by virtue of its linkage to several ongoing projects in the SAR watershed and elsewhere in southern California, as described in Section 18.

16. Nature, Scope, and Objectives of the Project

The objective of this proposed project is to define and model the ecological mechanisms underlying the FIB patterns illustrated in Fig. 1, and to determine if these patterns are reproducible across different sub-drainage sites and for different times of the year. Specifically, we will focus on the following observations (inspired by the results presented in Fig. 1, and other monitoring data not shown) that appear to be at odds with the standard build-up/wash-off paradigm:

- Rainfall of any amount (from trace to significant storms) triggers at least an order of magnitude increase in the FIB concentration over background levels (referred to here as "FIB events").
- FIB events last for approximately 10 days, where after FIB concentrations decrease one or more orders of magnitude to background levels.
- The duration of FIB events appears to be the same (~10 days) whether they are triggered by a single trace rainfall, or a multi-day storm.
- The ratios of the different indicator organisms vary by FIB event, and over longer periods (e.g., months) in the base flow.

The field and modeling studies proposed below are intended to determine: (1) The ecological processes that underlie the patterns described above (*Ecology Studies*). (2) If these patterns are are the same across different types of sites and at different times within the same site (*Variability Studies*). (3) If these patterns can be predicted through the coupling of ecological and transport models (*Modeling Studies*). Our project will concentrate on three different sites along an inland-to-coastal gradient that collectively represent a diversity of land-use and channel types. A description of the SAR watershed, and field sites within the SAR watershed, follows

SAR Watershed. The SAR watershed encompasses about 2,670 mi^2 of the densely populated coastal area of southern California between Los Angeles and San Diego, and includes the Santa

Ana River drainage basin and a few small streams near the coast that discharge into the ocean (Fig. 2). In 1990 the land use in the SAR watershed was 32% urban, 11% agricultural, and 57% undeveloped. In 1995 there were about 340 animal confinement facilities and more than 340,000 animals (primarily dairy cows) located primarily in the area drained by Chino and Cucamonga Creeks to the north of Prado Dam.

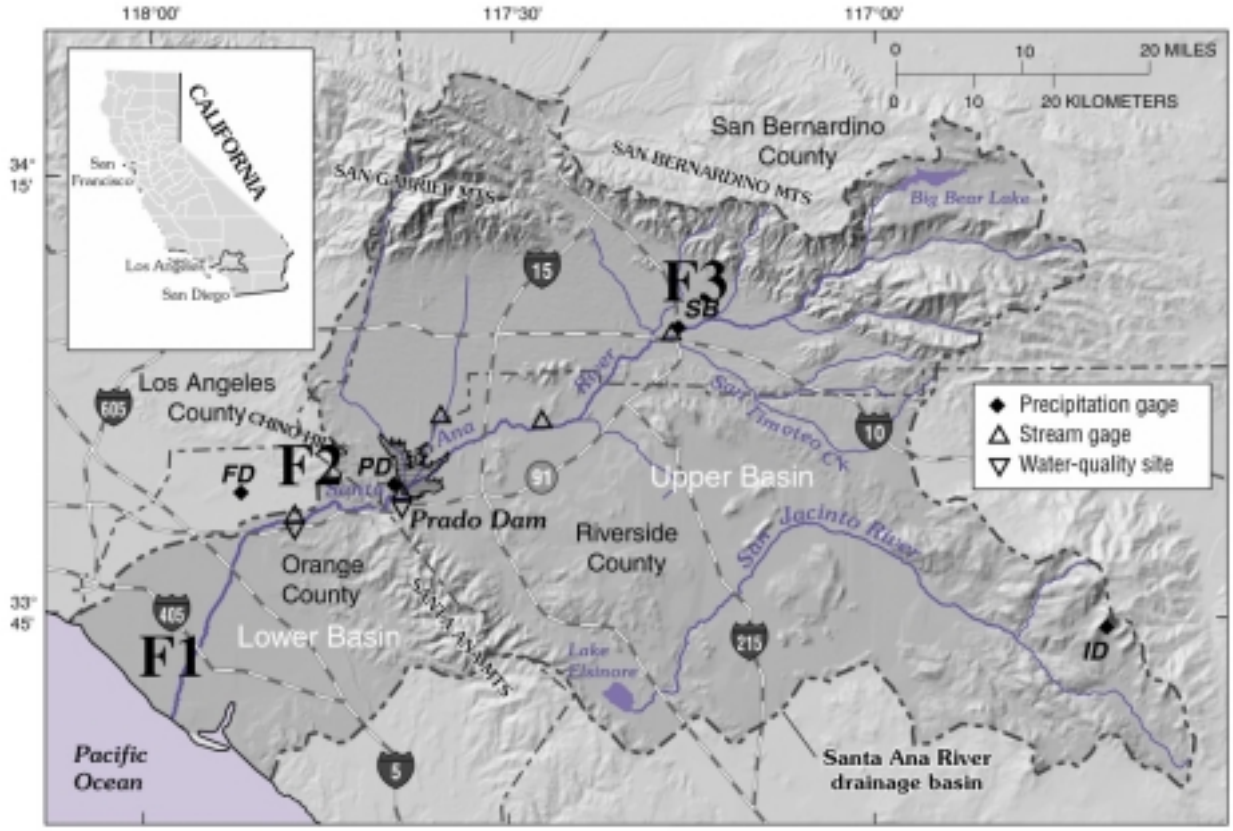

Fig. 2. A map of the SAR watershed. Proposed field sites are indicated by number: F1. Fountain Valley Channel, F2. Imperial Highway, and F3 Warm Creek.

Flow in the watershed is extensively managed for flood control and drinking water supply. Base (dry weather) flow in the SAR is maintained by discharges of treated municipal sewage from treatment plants in the Counties of San Bernardino and Riverside. Under all but the most extreme storm conditions, water in the SAR does not make it to the ocean outlet in Huntington Beach. Instead, flow downstream of Prado Dam, more than 200,000 acre-feet annually, is diverted by the Orange County Water District (OCWD) for groundwater recharge near the Imperial Highway Site (F2 in Fig. 2). Water pumped from the Orange County groundwater aquifers is the primary source of potable water supply for about 2 million people (OCWD, 1996). Most precipitation in the area falls between November and March (US Army Corps of Engineers, 1994), and hence flow in the SAR is typically greatest during this time period. Prado Dam is operated according to a complex set of procedures intended to minimize flood damage in the coastal plain, and to maximize availability of surface water for groundwater recharge by OCWD. Numerous studies have been conducted in the SAR watershed to characterize surface

water quality[2] , and groundwater recharge operations[3] . Beginning in 1997, the Santa Ana Basin was designated a NAWQA study unit, and several of the proposed field sites are part of the ongoing NAWQA program (http://ca.water.usgs.gov/sana_nawqa). The field sites are described next.

- *F1 Fountain Valley Site*. This site is located in a highly urbanized area on the coastal (southern) edge of the SAR watershed, near the intersection of the SAR and Pacific Coast Highway. The site drains 600 ha of commercial/industrial areas (40%), residential areas (45%), and parks and schools (15%) in the Cities of Fountain Valley and Huntington Beach. This site has been the location of historical water quality sampling by UCI personnel, as part of a EPA-STAR project that will be ending August 2003 (see Section 18).
- *F2 Imperial Highway Site*. This site is about 11 miles downstream from Prado Dam, at the diversion structure used to direct flow from the SAR channel into groundwater infiltration basins operated by the OCWD. Water quality and quantity at this site are affected by a combination of runoff from urban areas downstream from Prado Dam; runoff from areas upstream from Prado Dam; and, during the later part of the rainy season, runoff from previous storms stored behind Prado dam. The site has a permanent gaging station, has been the subject of extensive nutrient, pesticide, and heavy metal testing (e.g., Izbicki, 2000), and has a TC time series stretching back several decades.
- *F3 Warm Creek Site*. This site, which is a NAWQA urban indicator site, is located in the City of San Bernadino, near the northern edge of the SAR watershed. The site drains 11 mi^2 of residential, commercial, and industrial land uses. The USGS has operated this site as a continuous gaging station since October 1974, and 0.1 mi upstream on natural channel from February 1964 to September 1972. As part of the NAWQA program, this site is sampled 8 times per year.

Ecological Studies (Time Frame: 9/1/03 to 8/30/05, Lead Investigator: T. Holden)

A series of microbial ecology studies are planned to characterize the FIB communities in sediment and water samples collected from the three sites, and how these communities respond to storm events. In particular, the following hypotheses will be tested:

- *Hypothesis H1: The observed increase in FIB concentrations in runoff during, and after, storm events is accompanied by species shifts, perhaps reflecting shifts in the sources of these organisms (e.g., from sewage to re-growth) .*

- *Hypothesis H2: FIB communities in the sediments and water column will be similar during dry weather periods (reflecting the importance of ecological processes), and different during wet weather periods (reflecting transport controlled conditions).*

To test these hypotheses, water column and sediment samples will be collected from each of the three sites, at two different sampling frequencies: (1) hourly during the course of several storms,

[2] J. Izbicki, G. Mendez, C. Burton (2000) "Stormflow chemistry in the Santa Ana River below Prado Dam and at the diversion downstream from Imperial Highway, southern California, 1995-98", USGS Water Resources Investigations Report 00-4127; C. Burton, J. Izbicki, K. Paybins (1998) "Water quality trends in the Santa Ana River at MWD Crossing and below Prado Dam, Riverside County, California: USGS Water Resources Investigations Report 97-4173; Leecaster et al. (2002) Water Research, v. 36: 1556; Reilly et al. (2000) Ecological Engineering, v. 14:33; Ding et al. (1999) Chemosphere v.39:1781

[3] Williams (1997) J. of Hydrology, v. 201:230; Matthews (1991) Water Resources Bulletin, v. 27:841

and (2) four-times-weekly over a period of six months, commencing with the start of the storm season in January '04. Sediment and water samples from these sampling events will be analyzed for FIB (Colilert/Enterolert) to quantify the culturable bacteria that score positive on these tests (see Variability Section). Portions of the sediment and water samples will be preserved, and shipped Fed/Ex to UCSB where the microbial communities will be characterized by several methods including molecular (based on rRNA genes) fingerprinting of enterococci bacteria. .

Data collected from these experiments will allow us to correlate changes in the concentration of FIB groups (i.e., TC, FC or EC, and ENT) to shifts in the dominant species represented in each group (*Hypothesis H1*). Referring back to Fig. 1, for example, it is possible that merely changing the sediment water chemistry by trace rainfall events is sufficient to induce blooms of EC and ENT. Culture-independent fingerprints collected from this study will shed light on the degree to which FIB microbial communities in the sediment and water column are similar, how that similarity changes in response to storm events, and how these trends vary by site within the SAR watershed (*Hypothesis H2*).

Variability Studies (Time Frame: 9/1/03 to 8/30/05; Lead Investigator: S. Grant)

The goal of the variability studies is to determine if the occurrence patterns of FIB observed for the Fountain Valley site (FIB data in Fig. 1; labeled F1 in Fig. 2) are reproducible across different sites within the SAR watershed, and at different times at a single site. To this end, the three field sites described above will be sampled over two different time scales: (a) four times weekly for six months, (b) hourly over the course of several storms. In addition, we will incorporate into our analysis data from an ongoing monitoring program at the Imperial Highway site, where the Orange County Health Care Agency has been collecting samples for several decades. The sampling design is intended to test the following hypotheses:

- *Hypothesis H3: The occurrence patterns of FIB observed at the Fountain Valley Site are not unique to this one location, but rather are reproduced at multiple urban sites in the SAR watershed.*
- *Hypothesis H4: The occurrence patterns of FIB observed at the Fountain Valley Site are not unique to this one six month period, but rather can be reproduced at the same location over several different years.*
- *Hypothesis H5: Because FIB concentrations appear to quickly attain a constant value during a given storm, regardless of storm intensity (see Fig. 1), loading of FIB pollution from urban landscapes depends primarily on runoff volume.*
- *Hypothesis H6: Because peak FIB concentrations do not appear to decrease with subsequent storms (see Fig. 1), the annual load of FIB shed from a sub-drainage will scale with annual precipitation.*

The four-times weekly data collected from the three SAR watershed sites, together with flow and rainfall records provided by the USGS, will permit us to determine if the patterns illustrated in Fig. 1 are reproducible across sites (*Hypothesis H3*). Furthermore, by choosing the Fountain Valley site (F1 in Fig. 2) as one of our sampling locations, we can compare FIB occurrence patterns observed in 2001 (see Fig. 1) with observations in 2004 (*Hypothesis H4*). To determine how FIB loading is related to runoff volume (*Hypothesis H5*) and how it responds to decadal shifts in weather patterns (e.g., positive and negative Pacific Decadal Oscillations, *Hypothesis*

H6), FIB loading will be computed from multi-decade records of weekly FIB measurements and stream gage information at the Imperial Highway site. Many of our sampling efforts, particularly those coinciding with storm flow events, will be carried out side-by-side with USGS personnel, who will be quantifying stream flow and analyzing samples for nitrogen (nitrate,nitrite, ammonia, and organic nitrogen), phosphorous (phosphate and orthophosphate) and DOC.

Modeling Studies (Time Frame: 9/1/04 to 8/30/06, Lead Investigator: B. Sanders)

The modeling studies will incorporate lessons learned from the field studies into a flow and transport model suitable for inclusion into BASINS. Specifically, the modeling effort will test the following hypothesis:

- *Hypothesis H7: The occurrence patterns of FIB in streams can be predicted once the dominant ecological processes are incorporated into flow and transport models.*

This hypothesis will be tested by coupling a FIB ecology model to a multi-dimensional hydrodynamic solute and particle transport model developed by B. Sanders[4]. The details of the FIB ecology model will obviously depend on the conceptual picture that emerges from the field-work described above. For example, if the data suggest that trace rainfall triggers blooms of specific enteric bacterial species, then we will incorporate growth rate expressions for FIB species into the flow and transport model. The growth rate constants may, in turn, be coupled to the concentration of growth-limiting nutrients (e.g., nitrogen or DOC) through a separate set of transport equations. Alternatively, if the ecology studies suggest that growth of bacteria at the sediment-water interface elevates dry-weather FIB levels in the water column, then we will introduce a sediment module that incorporates appropriate growth rate and mass transfer expressions. As models for FIB ecology are developed and mathematically formulated, they will be tested against historical and recently collected FIB occurrence patterns measured at the three field sites (*Hypothesis H7*).

17. Methods, Procedures, Facilities

Ecology and Variability Studies. Immediately after collection, all water samples are placed in an ice-filled cooler (to shield the samples from sunlight and slow die-off), and transported to the laboratory at UCI for bacterial and physical analyses within 6 h. At the lab, 250 mL aliquots of the sample are tested for salinity /TDS and pH (ThermoOrion 162A conductivity meter and ThermoOrion 720A pH meter, respectively). Sixty mL of each sample is analyzed for turbidity using a HF scientific DRT-15CE Turbidimeter. Approximately 20 mL of each sample is tested for FIB (TC, EC, and ENT) using the defined substrate tests Colilert and Enterolert by IDEXX. The laboratory at UCI is a fully equipped to process large number of water quality samples, including four quanti-tray sealers, four constant temperature/humidity incubators, and two walk-in constant temperature rooms. Bacteria will be eluted from sediment samples by either sonication or blending[5], the latter of which has been used extensively in soils to liberate bacteria

[4] Arega, F. and Sanders, B.F. (2003) Dispersion Model for Tidal Wetlands, ASCE Journal of Hydraulic Engineering. (submitted).

[5] Holden, P. A., L. E. Hersman and M.K. Firestone. 2001. Water content mediated microaerophilic toluene biodegradation in an arid vadose zone. Microbial Ecology 42(3):256-66.

for microscopy and culturing. The number of dilutions required for accurately quantifying FIBs in both the overlying water and sediment samples will be estimated by preliminary assays at the beginning of this study. To assess if community shifts are occurring in sediments and if, perhaps related, shifts are visible in the overlying water, we will analyze terminal restriction fragment length polymorphisms (T-RFLP) of PCR-amplified rRNA genes in the sediment and water bacterial communities. Fingerprinting is intended to screen for shifts in the total community (using eubacterial primers) and in the enteroccus community (using enterococcus-specific primers). Community shifts before and after a storm may indicate selective growth of populations that effectively increase the FIB signal. Documenting community shifts is the necessary first step preceding a later, perhaps more quantitative, study to track the changes of specific populations which may be related to either their growth or changed culturability, or both. To document shifts in the whole bacterial communities, DNA will be extracted from water and sediments as we have done previously[6,7,8] using eubacterial primers (fluorescently-labeled forward), PCR products purified and restricted using selected restriction enzymes, and restriction fragment lengths determined as before. A separate PCR reaction, using *Enterococcus* specific primers[9] will be conducted for all samples and T-RFLP analysis performed to detect shifts in the *Enterococcus* communities. Importantly, the two primer sets (eubacterial and *Enterococcus*) are specific to different ribosomal subunit genes (16S and 23S, respectively). We favor this pairing of breadth and depth in our community assays because this enables us to couch our interpretation of *Entercoccus* community shifts within the context of the rest of the eubacterial community which may be undergoing parallel shifts in composition.

Modeling studies. The Hydrologic Simulation Program – Fortran (HSPF) in BASINS routes flow and water quality using RCHRES, a hydrologic routing model for river reaches and reservoirs. Lacking a comprehensive understanding of bacterial ecology in streams, these models have only accounted for die-off and therefore cannot explain the variability presented in Figure 1. We will use a much more detailed model for ecology of bacteria in streams which includes a coupled set of continuity, momentum, solute, and particle transport equations. The continuity and momentum equations are depth-integrated and solved using a finite volume scheme that was specifically designed to address wetting and drying of irregular topography[10]. Streams of the Santa Ana River watershed (at least where monitoring is proposed) consist of rectangular or trapezoidal channels that at full bank flow could easily be characterized by a 1D model. However, during dry weather periods flow does not wet these channels from bank to bank and instead either snakes along the bottom or includes regions of braided flow. For this reason and

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[6] LaMontagne , M.G., Michel, F.C. Jr., . Holden, P.A. and C.A. Reddy. 2002. Evaluation of extraction and purification methods for obtaining PCR-amplifiable DNA from compost for microbial community analysis. Journal of Microbiological Methods 49:255-64.

[7] LaMontagne, M.G., J. P. Schimel and P. A. Holden. 2003 Comparison of subsurface and surface soil bacterial communities in California grassland as assessed by terminal restriction fragment length polymorphisms of PCR-amplified 16S rDNA. Microbial Ecology (in press)

[8] LaMontagne, M. G. and P. A. Holden. 2003. Comparison of free-living and particle-associated bacterial communities in a coastal lagoon. Microbial Ecology (in press).

[9] Frahm, E. and U.Obst. 2003. Application of the fluorogenic probe technique (TaqMan PCR) to the detection of Entercoccus spp. and Escherichia coli in water samples. J. Microbiol. Meth. 52: 123-131.

[10] Bradford, S.F. and Sanders, B.F. (2002) Finite-Volume Model for Shallow-Water Flooding of Arbitrary Topography, ASCE Journal of Hydraulic Engineering. Vol. 128, No. 3, pp. 289-298.

because sediment wetting by dry-weather flow may be important to the bacterial ecology, the 2D formulation was selected. The solute transport model is depth-integrated and could be used to predict the distribution of physical parameters such as temperature and salinity, nutrients such as nitrate, phosphorous, and TOC, and bacterial population; and transport equations are solved using the same finite volume scheme used to solve the flow equations. One of the key advantages of this model is its use of physically meaningful mixing coefficients that do not require site specific tuning[4]. Calibration is limited to minor adjustments of the two key parameters required by the model: bed elevation and bed roughness data. These parameters (which vary across the spatial domain of the model) are adjusted within their uncertainty to bring velocity and discharge predictions in line with measured values, and in our experience modeling mixing in tidal wetlands with channel networks and oscillating flow fields, dispersion is accurately predicted using physically meaningful mixing coefficients once a detailed description of the flow is obtained. This requires a careful mapping of the bed elevation at each of the study sites, which is planned component of this investigation. Finally, the particle transport model is three-dimensional and includes a random-walk approach to simulate eddy diffusion[11]. The three-dimensional velocity field is recovered from the depth-averaged flow prediction under the assumption of a von Karman-Prandtl logarithmic velocity profile. The three-dimensional particle formulation is not subject to the Lagrangian time scale restriction associated with the solute transport model, and by virtue of resolving the third dimension of transport it may be an attractive alternative to depth-integrated solute transport for some components of this system.

The computational demands of seasonal time scale predictions are immense with high-resolution multidimensional models. To alleviate this problem, we will apply the model at the sub-catchment scale and not the watershed as a whole. For long time scale predictions, we will also create a database of multi-dimensional flow predictions for a range of steady stream flow rates, and then model the flood hydrodynamics as a series of steady states as is done in many flood models. The transport model will then utilize the database of flow predictions to aid in the prediction of bacteria levels, which will greatly expedite the computational procedure. Stage and discharge will be measured by USGS personnel at each of the field sites so appropriate flow conditions will be used by the model. Loads of bacteria into the channel will be estimated either by direct measurement or by wash off coefficients as is done in BASINS. Once we are successful at predicting fecal indicator bacteria in streams using this approach, a cross-sectionally averaged formulation suitable for inclusion in HSPF will be developed.

18. Related Research

The proposed project will greatly complements, but does not overlap with, already published studies on the watershed sources and transport of FIB. Published articles tend to focus on either the soil and water column ecology of FIB[12], computer predictions of FIB shedding[13] , source tracking exercises, but not the linkages between these topics, as proposed here. The proposed

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[11] Dimou, K.N. and Adams, E.E. (1993) A Random-Walk, Particle Tracking Model for Well-Mixed Estuaries and Coastal Waters," Estuarine, Coastal and Shelf Science, 37, pp. 99-110.

[12] Fujioka R et al., (1999) "Soil:the environmental source of *E. coli* and enterococci in Guam's streams", J. Applied Microbiology, 85:83S-89S.

[13] STY, WL Chen (2002) "Modeling the relationship between land use and surface water quality", J. Environmental Management 66:377-393; Scarlatos, P.D. (2001) "Computer modeling of fecal coliform contamination of an urban estuarine system" 44:9-16.

project also complements many ongoing, or recently completed studies, in the SAR watershed, coastal region near the SAR outlet, and in watersheds elsewhere in the southern California Bight. Synergistic research at Huntington Beach and the surrounding area is funded by the National Water Research Institute, local agencies (including Santa Ana Regional Board, County of Orange, Coastal Cities, and Orange County Sanitation District), NSF, and the US EPA STAR program. Collectively, these studies aim to

- Define the relationship between pathogens and indicators;
- Measure the export rate of indicators from coastal outlets of the Santa Ana River and Talbert Marsh;
- Evaluate the efficacy of best management practice (BMP) strategies for reducing the shedding of fecal pollution from the urban landscape (e.g., dry weather diversions); and
- Characterize the error rates associated with current public notification protocols.
- Develop new-generation multi-dimensional hydrodynamic models for prediction of flooding and drying over natural topography.
- Link hydrodynamic model with solute transport and particle transport layers for tracking pollutant dispersal, particularly in tidally influenced coastal wetlands

Additional work, sponsored by the University of California Marine Council aims to understand the role that tidal salt water marshes play in modulating (either positively or negatively) the concentration of fecal pollution in coastal waters. The UCMC project supports a collaborative effort consisting of wetland ecologists, surface water hydrologists, physical oceanographers, and environmental engineers.

19. Training Potential

Stipend and research support for two graduate students, and up to thirty undergraduate students (for episodic field data collection) is budgeted, to assist the three co-PIs in the collection and analysis of field data, and modeling effort. This project will be beneficial to graduate students by providing a highly interdisciplinary research environment in which ecologists, environmental engineers, hydrologists, and USGS personnel will work together to address the same basic questions. We will have quarterly project meetings of participating students, faculty, and staff to plan research activities, share data, conduct joint analyses, and discuss interpretations.

20. Statement of Government Involvement

Personnel from the USGS office in San Diego (including Clinton Church and John Izbicki) will be involved in every stage of the planning and execution of the field experiments, and analysis of field data. Specifically, the USGS will make available historical gaging data on the SAR, digital elevation maps of the field sites for inclusion in the hydrodynamic model, will anticipate storm events for the storm sampling studies using Doppler radar, and will make available nutrient data collected in parallel with our FIB studies (see letter of collaboration).

21. Information Transfer Plan

The results of this study will be transferred in three ways: (1) The generation of manuscripts for submission to peer-review journals that describe the field and modeling results. (2) Quarterly briefings with stakeholders in the watershed (consisting of County, City, OCWD, US Army Corps, and the USGS) in which field sampling plans and preliminary results will be shared and discussed. (3) The generation of linked transport and FIB ecology models suitable for inclusion into the BASINS system.

Distribution and toxicity of sediment-associated pesticides in the Sacramento River watershed.

Basic Information

Title:	Distribution and toxicity of sediment-associated pesticides in the Sacramento River watershed.
Project Number:	2003CA57G
Start Date:	9/30/2003
End Date:	9/29/2005
Funding Source:	104G
Congressional District:	9th
Research Category:	None
Focus Category:	Agriculture, Non Point Pollution, Sediments
Descriptors:	None
Principal Investigators:	Donald Paul Weston, Michael j Lydy, Chris Ingersoll

Publication

Distribution and toxicity of sediment-associated pesticides in the Sacramento River watershed.

Principal Investigator - Donald Weston, University of California, Berkeley, CA
Subaward PI - Michael Lydy, Southern Illinois University, Carbondale, IL
USGS Collaborator - Chris Ingersoll, U.S. Geological Survey, Columbia, MO

Statement of critical regional or State water problem:

Due to increasing regulatory restrictions on organophosphate (OP) insecticides, many sectors of the agriculture industry in the U.S. are increasingly relying upon pyrethroids. For example, the acreage of orchards in California's Central Valley to which pyrethroids were applied increased 7-fold from 1992 to 1998 (Epstein et al., 2000), and use has increased further since then. In addition, recent national prohibitions on the use of certain OPs in home and garden products has led pesticide manufacturers to promote products containing pyrethroids as alternatives, thus leading to dramatic increase in pyrethroid usage in residential areas. Pesticide monitoring programs in California have traditionally targeted the water column for chemical analysis of pesticide residues and toxicity testing. While this approach has been appropriate for the relatively water-soluble OP insecticides that have been heavily used throughout the 1990s, it makes little sense for more hydrophobic compounds such as organochlorines and pyrethroids. Pyrethroids have an affinity for particles that is 100-1000 times greater than the OPs, and thus will largely be found in the sediments, except perhaps immediately after and in close proximity to their application. Thus, we are presently confronted with a dilemma in that both agricultural and urban pesticide users are switching, in fact are being encouraged to switch, to pyrethroid pesticides, yet we know little about the environmental fate and effects of these compounds, there is remarkably little sampling of the sediment matrix where residues would be found or toxic effects manifested, and suitable analytical protocols are available but not yet widely used. The adverse effects of OPs on aquatic ecosystems are well documented (e.g., Werner et al., 2000) and thus the regulatory pressure to switch to pyrethroids is understandable, but we run the risk of trading a known and well monitored water column toxicity problem for an unknown and poorly monitored sediment toxicity problem.

Over 30 water bodies in California's Central Valley have been 303(d) listed because of pesticide contamination, especially diazinon and chlorpyrifos, and state agencies are currently developing TMDLs for many of these water bodies. Sediment-bound pesticides have not been the cause for any 303(d) listings, probably a result of the fact that minimal sampling has been done for sediment-associated pesticides in the Central Valley. However, attention to sediments in impaired water bodies is all the more important given that the reduction in OP inputs necessary to achieve de-listing is likely to be accompanied by an increased reliance on pyrethroids. Consideration of sediment toxicity is necessary to insure that remedial measures and de-listing decisions are based on information that considers all environmental matrices, and does not treat the mere transfer of toxicity from the water column to the sediments as a success. For this reason, the proposed study targets 303(d) listed water bodies whenever feasible.

Statement of results or benefits:

Environmental managers are coming to the realization that the water quality improvements that have been achieved by encouraging pesticide users to switch from OPs to pyrethroids may have hidden costs. The current difficulty is that environmental managers, and in fact most stakeholders, have an awareness of emerging pyrethroid use and potential for environmental impacts, but completely lack data by which to assess these risks. This study will provide such data to all stakeholders, and by doing so, will alleviate the current uncertainties and promote environmentally protective selection of agents and methods for pest control.

The water quality issues associated with diazinon and chlorpyrifos which have resulted in the 303(d) listing of many water bodies were not well documented until the 1990's, 40 years after introduction of these pesticides and after about 4 million pounds were being applied annually by farmers in California (plus unquantified urban usage). As pyrethroids increasingly replace the OPs, we believe it is prudent to initiate the environmental studies early in that process. Current agricultural pyrethroid usage in California is now on the order of 250,000 lb annually, and only

in the past year has data become available on concentrations of multiple pyrethroids in Central Valley sediments (to a large degree, from our own studies). By studying the environmental fate and effects of this pesticide class now, appropriate management measures can be initiated early, and 20 years from now environmental management agencies and pesticide users will hopefully avoid the same predicament that currently exists with the OPs. We believe both environmental managers and pesticide user groups would benefit by obtaining this type of environmental data as pyrethroid use is emerging, rather than playing "catch-up" after their use has become widespread and application practices have become entrenched.

As discussed in more detail below, we are in the midst of a study on pyrethroid distribution in aquatic sediments in areas of intensive pyrethroid use within the San Joaquin River watershed, California, as well as toxicity testing of these sediments. We have submitted a proposal to the PRISM program of the State of California to extend this work to the Sacramento River watershed. Taken together, these two studies will provide a good picture of the distribution of pyrethroids and sediment toxicity in California's Central Valley. While these investigations will help meet the immediate information needs of environmental managers as to whether an aquatic environmental problem exists with current pyrethroid use practices, these studies do little to increase understanding of the basic ecotoxicology of pyrethroids. The study proposed herein is intended to provide this fundamental understanding, thereby giving us greater predictive capability as pesticide use patterns change, help to better focus subsequent investigations, and provide data that will be not only of regional, but of nationwide value.

Nature, scope and objectives of the project, including a timeline of activities:

The proposed work is an extension of an on-going study emphasizing the San Joaquin River watershed in which we have proven the analytical feasibility of sediment analysis for pyrethroids and have shown toxicity to benthic invertebrates at sites having elevated concentrations (You et al., in review). Based on the results of that study, it is our hypothesis that pyrethroid and organochlorine insecticides will be present in the sediments at detectable levels at the majority of sites which we will sample in the Sacramento River watershed.

We have submitted a proposal to the PRISM program administered by California's State Water Resources Control Board. The PRISM funding, as well as additional matching funds from UC Berkeley and Southern Illinois University, will be used to meet the match requirement of this USGS grant. The PRISM grant will allow us to: 1) collect sediments from water bodies draining areas of high agricultural pyrethroid use, as well as urban-dominated water bodies, and test these sediments for pesticide residues and acute toxicity; 2) manipulate pyrethroid concentrations of sediments in the laboratory to determine levels necessary to cause lethal and sublethal toxicity; 3) determine persistence of residues in farm and urban soils in order to guide mitigation efforts.; and 4) promote awareness of the need for monitoring pyrethroids and other sediment-associated pesticides and demonstrate the analytical feasibility of doing so. All work described above in the PRISM proposal will be done (if funded) regardless of whether USGS funding is provided.

The proposed two-year study is highly integrated with the PRISM work, but allows far more work to be done on pyrethroids, and particularly on more basic aspects of their toxicology. This study will have the following objectives:

1) Conduct bioaccumulation and toxicokinetics studies that examine how pyrethroids are processed by benthic invertebrates.
2) Determine the potential enhancement of pyrethroid toxicity by piperonyl butoxide (PBO), a synergist included in some pesticide formulations and known to be present in Central Valley surface waters.
3) Examine possible interactions between pyrethroids and organochlorines that may modify the expected independent toxicity of the compounds.
4) Collect and analyze native benthic invertebrates from the PRISM stations for tissue pyrethroid concentrations.
5) Run chronic toxicity tests on selected sediment samples collected as part of the PRISM project. Dr. Chris Ingersoll, a USGS Scientist located at the Columbia, MO laboratory, will conduct this testing.

6) Measure changes in bioavailability (toxicity tests) and aqueous desorption rates (Tenax beads) of pyrethroids in field-collected soils over time.

Table 1 provides a timeline for the proposed studies. Since the PRISM and USGS work are so highly integrated, Table 2 illustrates which components of the work will be funded by each program. While all components of the work are discussed in the text below, greater detail is provided for those aspects for which USGS support would be required.

Table 1. Timeline for proposed studies.

	2003][2004][2005]
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Bioaccumulation & toxicokinetics	**********
PBO synergist studies	**********
Pesticide interactions	**********
Field sampling	**********
Chronic toxicity bioassays	**********
Bioavailability/desorption studies	**********
Data analysis & manuscript prep.	******

Table 2. Illustration of which project components discussed in the text of this proposal would be supported by State of California (PRISM) and the USGS/NIWR program.

STUDY ELEMENT	PRISM-SUPPORTED	USGS/NIWR-SUPPORTED
Bioaccumulation and toxicokinetics	None	Uptake and depuration kinetics in two species.
PBO synergist studies	Determination of pyrethroid toxicity in the absence of PBO (3 pyrethroids spiked into 3 sediments of varying organic carbon content).	Modification of toxicity by PBO, and manipulation of PBO concentration and exposure period.
Pesticide interactions	None	Pyrethroid and organochlorine interactions.
Field sampling	Collection of sediment at approx. 16 agriculture and urban-dominated sites in the Sacramento River watershed, and analysis for pyrethroids and organochlorine residues.	Collection of resident invertebrates at these same sites, and analysis of their tissues for pyrethroid residues. Addition of three more urban sites for analysis of sediment residues.
Toxicity testing	Acute toxicity testing of above sediments with H. azteca and C. tentans.	Chronic toxicity testing of a subset of the sediments with H. azteca.
Persistence, bioavailability, and desorption studies	Collection of soils from 3 farms at multiple time points before and after pyrethroid application. Analysis of these soils to determine persistence of pyrethroid residues.	Using these same farm soils, examine changes in the bioavailability and aqueous desorption of pyrethroid residues over time since chemical presence alone may not be an adequate measure of risk.

Methods, procedures and facilities:

Bioaccumulation and toxicokinetics

Toxicokinetics is the study of the rate processes involved in uptake, distribution, metabolism and elimination of a toxic chemical in an organism. This information is critical when judging the potential for toxicity and bioaccumulation of chemicals, as well as the potential for trophic transfer of toxic substances to predators. Conventional wisdom is that pyrethroids have little potential to bioaccumulate through the food chain because of rapid metabolism. However, aquatic organisms tend to metabolize pyrethroids much slower than warm-blooded terrestrial organisms. Even fish, which might be expected to metabolize them rapidly, will retain parent compound for several days (Coats et al., 1989). Biotransformation capabilities are even weaker in some invertebrate taxa such as molluscs and some annelids, and we suspect persistence of pyrethroids in their tissues may provide a route for uptake of residues by their predators.

This component of the project will determine toxicokinetic parameters such as sediment uptake clearance coefficients (k_s), elimination rate constants for both parent compound (k_{ep}) and metabolites (k_{em}), the biotransformation rate (k_m), biological half life ($t_{1/2}$) and bioaccumulation factors (BAF). Two invertebrate species that differ widely in biotransformation capabilities will be used. Previous studies in our laboratory have shown that Chironomus tentans readily metabolizes insecticides, while Lumbriculus variegatus, does not have this capability.
The toxicokinetics work will be conducted using sediment exposures following the methods of Landrum et al. (1991). Uptake clearance rates will be measured by placing animals into sediment spiked with a radiolabelled pyrethroid (either ^{14}C-permethrin or ^{14}C-esfenvalerate). Specific sampling times will be determined in preliminary studies for each of the test species, but a general plan is to collect samples at 0.5, 1, 2, 4, 6, 8, 10 days after sediment exposure. At each sampling time, levels of radioactivity in animals, water and sediment will be analyzed. Biotransformation potential will be determined by fractionating tissue activity into parent or metabolites by thin layer chromatography (TLC) or by high performance liquid chromatography (HPLC) using the methods of Lydy et al. (2000).

Elimination rate constants will be measured by placing contaminated test organisms into uncontaminated sediment with the overlying water replaced 3-4 times per day. Triplicate samples of organisms will be withdrawn at each sampling time. Duration and frequency of sampling will both depend on elimination rate. Toxicokinetic parameters will then be determined using a two-compartment model (Lydy et al. 2000). This model incorporates uptake from the environment, rate of biotransformation from parent compound to metabolites and elimination rates for both the parent compound and metabolites. Data will be collected so that the uptake and elimination phases can be modeled simultaneously.

Bioaccumulation factors will be estimated from the kinetics using the equation:

$$BAF = \frac{C_p}{C_s} = \frac{k_s}{(k_{ep} + k_m)}$$

where:, C_p = conc. of parent compound in animal; C_s = conc. of chemical in sediment; k_s = sediment uptake clearance coefficient; k_{ep} = parent elimination rate constants; and k_m = biotransformation rate constant.

To properly evaluate a BAF for a metabolized compound, both the elimination rate of parent compound (k_{ep}) and loss rate via biotransformation (k_m) must be considered. The biological half-lives of parent compound ($t_{1/2p}$) and metabolites ($t_{1/2m}$) will also be determined. A subsample of organisms will be taken for lipid analysis. Lipid levels in the organisms will be determined by spectrophotometry using the method of van Handel (1985).

PBO synergist study

Piperonyl butoxide (PBO) is a synergist used to enhance the insecticidal properties of pyrethroid insecticides. The function of PBO is to inhibit metabolic degradation of the insecticide by the target pest species. With metabolic detoxification inhibited or completely stopped, the toxic potency of the pyrethroid is increased. PBO is included in many pyrethroid

pesticide formulations, and residues of the compound are detectable in surface waters of California (K. Kuivila, pers. comm.). The demonstration of PBO in surface waters raises the possibility that PBO may be having the same synergistic effect on aquatic non-target species as on the pest for which was initially applied, and suggests that literature estimates of pyrethroid toxicity may underestimate the risks since they do not include the possibility of a co-occurring synergist.

Under the PRISM-funded study, we will be spiking sediment with three pyrethroids (permethrin, esfenvalerate, lambda-cyhalothrin in independent trials) and determining their toxicity to an aquatic invertebrate. The species used is likely to be Hyalella azteca, but we are awaiting the results of on-going relative sensitivity studies in our laboratory. Parameters such as LOEC and LC_{50} will be determined. With USGS support, we will extend this work to include examination of the potential enhancement of pyrethroid toxicity by PBO using PBO concentrations typically detected in surface waters in California.

After determining LC_{50} values for the pyrethroids in the absence of PBO, we will conduct additional trials manipulating both the concentration range of PBO and the duration of PBO pre-exposure prior to pyrethroid exposure. We anticipate doing 10-day tests with sediment-associated pyrethroids and regular water changes containing PBO, with varying periods of PBO exposure prior to introduction of pyrethroid-contaminated sediments. Information on environmentally realistic PBO levels in California surface waters is available from the USGS Sacramento office and will be used to establish appropriate PBO concentrations in these tests. We will study both simultaneous PBO/pyrethroid exposure, as well as vary the duration of an initial PBO pre-exposure in case synergism is best demonstrated by enzymatic inhibition before exposure to the insecticide. PBO and control exposures will be compared by calculating synergistic ratios (SR). A SR greater than 1 indicates that synergism has occurred.

$$SR = \frac{LC_{50}\,\text{compound}}{LC_{50}\,\text{compound + synergist}}$$

Pesticide interactions

Our understanding of interactions of pesticides in mixtures is generally limited and restricts our ability to predict impacts of environmental contamination. Little if any research has been conducted examining interactions among pyrethroid insecticides or between pyrethroids and organochlorine pesticides. There is significant potential for interactions as both pesticide groups are neurotoxins with similar modes of action. The objective of this study component is to investigate, for selected species, potential interactions among pyrethroids and among pyrethroids and organochlorines, and classify these interactions as additive, synergistic or antagonistic.

Mixture toxicity testing will be performed on C. tentans, and will be determined using a modified toxic unit approach (Pape-Lindstrom and Lydy, 1997). In the toxic unit (TU) model, a value of 1 TU is assigned to the LC_{50} value of each contaminant. A sum of the TU contributed by each component describes the toxicity of a mixture as follows:

$$TU_{sum} = \frac{C_{w_1}}{LC_{50_1}} + \frac{C_{w_2}}{LC_{50_2}} + \ldots + \frac{C_{w_i}}{LC_{50_i}}$$

where: Cw_i is the concentration of a chemical in a mixture and LC_{50i} is the LC_{50} for the respective component chemicals of the mixture from 1 to i. Empirically measured toxicity can then be compared to expected toxicity that is generated using LC_{50} values determined in tests of individual toxicants. When 50% mortality occurs at TU values lower than 1, the mixture is exhibiting greater than additive toxicity (synergism). Determination of less than additive toxicity (antagonism) is made when 50% mortality occurs at TU values greater than 1.

We will initially establish a LC_{50} for individual pesticides in the test sediment (bifenthrin, esfenvalerate, cypermethrin, permethrin, *lamda*-cyhalothrin, DDT, DDE). Acute toxicity testing will be conducted in static systems for 10 days. The LC_1, LC_5, LC_{15} and LC_{50} values will be determined for each pesticide using probit analysis. Acute toxicity tests with binary mixtures

will be conducted in a manner similar to the individual pesticide tests. Concentrations of each pesticide will be added at proportions of their respective LC_{50} so that the sum of concentrations of the pesticides are equivalent to five concentrations: 0.5 TU, 0.75 TU, 1.0 TU, 1.5 TU, and 2.0 TU. Actual mortality in mixtures will be compared to predicted toxicity assuming additivity.

Field sampling (including collection on resident species and chronic toxicity testing)

Sampling locations will be established early in the project through the PRISM project. Briefly, the State of California maintains a Pesticide Use Reporting (PUR) database which records all agricultural use of pesticides, and commercial urban uses. This database will be analyzed to determine the locations of greatest agricultural use of pyrethroids in the Sacramento River Watershed, and 11 sampling sites will be establish in watercourses which drain these areas. Sampling sites will be selected among these watercourses with the goals of: 1) obtaining broad regional coverage; 2) achieving diversity in the types of crops represented by the sampling sites; and 3) particularly targeting 303(d) listed waterbodies if they drain these high use areas. In addition, 5 creeks receiving urban runoff will be sampled around Sacramento, Yuba City and Chico. The agricultural sites will be sampled immediately after the time of greatest pyrethroid use (regionally varying depending on dominant crop, but usually summer) and again in February following winter rains. The urban sites will be sampled after the first rain event of the winter (usually November) and again at the end of the rainy season in April.

Surficial sediments (approx. upper 1 cm) will be collected at all sites and analyzed for a suite of 27 pesticides that are likely to be sediment-associated including an OP (chlorpyrifos), 19 organochlorines, and 7 pyrethroids. Pyrethroids analyzed will include bifenthrin, cyfluthrin, cypermthrin, deltamethrin, esfenvalerate, lambda-cyhalothrin and permethrin. The organochlorine pesticides that will be analyzed include *alpha*-BHC, *beta*-BHC, *gamma*-BHC, *delta*-BHC, heptachlor, aldrin, heptachlor epoxide, *gamma*-chlordane, *alpha*-chlordane, endosulfan I, DDE, diedrin, endrin, DDD, endosulfan II, DDT, endrin aldehyde, endosulfan sulfate, methoxychlor and endrin ketone. Analytical methods will follow those recently developed in our laboratory. A manuscript describing this protocol is in review by the Journal of Chromatography (You et al., in review).

The sediments will also be tested for acute toxicity using H. azteca and C. tentans, both species which are widely utilized for sediment toxicity testing. From this data set we will be able to establish the concentrations of pyrethroids occurring in aquatic sediments in regions of most intensive use within the Sacramento Basin, determine if sediments exceed toxic thresholds, and gather information on the relative sensitivity of these two species in order to guide future efforts.

USGS support will extend this PRISM-supported field work in three areas. First, it will permit sampling at three more urban sites in the vicinity of San Leandro, California. The urban sites sampled under PRISM are surrounded by intensively cultivated lands, and it has been found that urban streams may contain agricultural pesticides carried into urban areas by aerial transport, rather than used in the urban area per se. San Leandro, on the east side of San Francisco Bay, is highly urbanized but upwind of agricultural areas, given the prevailing winds of the region. Sampling in these creeks will help us determine if pyrethroid pesticides are found in urban areas due to local usage rather than agricultural influence, and will be a useful comparison for the other Central Valley sites.

Secondly, resident species, most likely chironomids and oligochaete worms both of which are common in our field sampling areas, will be collected from field sites when available, and analyzed for the 27 insecticides mentioned previously using a modification of the procedure used by the U.S. Environmental Protection Agency for their tissue monitoring programs (USEPA, 1980; 1986; 1996). These data on tissue residues in field-collected organisms are collected primarily to provide field validation of the bioaccumulation and toxicokinetic laboratory studies. The lab studies will be on very similar species (C. tentans and the oligochaete L. variegatus). We wish to determine if lab-derived BAFs for pyrethroids are comparable to those measured in the field, and to determine if ingestion of aquatic invertebrates under natural conditions could serve as a route of pyrethroid exposure to predators.

Thirdly, the U.S. Geological Survey Columbia Environmental Research Center (CERC) will conduct whole-sediment toxicity tests using methods described in ASTM (2001) and

USEPA (2000). A subset of sediments collected from the PRISM project will be examined for chronic toxicity (~8-10 samples). These sediments will be chosen based on two criteria; sediments must not show overt acute toxicity in the PRISM tests; and the sediments must contain detectable levels of pyrethroids determined analytically as part of this project. Briefly, 7-d old H. azteca will be exposed in 100 ml of sediment with 175 ml of overlying water in 300 ml beakers. Exposures will be conducted for 28 days with 4 replicates/treatment, and amphipods will be fed 1.0 ml YCT/day (1800 mg/L stock). Endpoints measured at the end of these exposures will be survival and growth (body length), and these data will be useful in determining whether chronic exposure to pyrethroids may represent a threat not addressed by the acute tests used for testing of the other sediment samples from this project.

Persistence, bioavailability and desorption:

Pyrethroids are applied to fields where they bind to plant materials or the surrounding soils. Some time may elapse between when this initial adsorption occurs, and when those particles are transported to aquatic systems, usually by runoff. In California, a period of about 4-5 months would be expected given, for example, pesticide usage in July and no appreciable rainfall until November/December. Under the PRISM study, we will be sampling at 3 farms (various crops) which had used pyrethroids, and monitoring the soils at multiple time points post-treatment for the presence of pyrethroid residues. USGS support will be used to measure relative changes in bioavailability and aqueous desorption rates of pyrethroids in field-collected soils as they age. Acute toxicity tests will be used as the measure of bioavailability, while desorption rates will be determined using Tenax beads. Soil will be taken from agricultural sites at 7, 30, 120, and 270 days post-application. The pyrethroid studied will be dependant upon site usage, but most likely will be permethrin or esfenvalerate. A subsample of the soil will be hydrated with moderately hard water and used for toxicity testing using a standard 10-d acute toxicity test will be conducted using H. azetca. Assuming the soil is toxic, a dilution series will be used to determine the nmber of toxicity units in the sample, and this parameter will be tracked over time to monitor temporal patterns of bioavailability. The remaining soil will be used for the desorption experiments. Desorption experiments will follow the methods of Cornelissen *et al.* (1997). Briefly, sediment and Tenax beads will be added with 50 ml water containing $HgCl_2$ to prevent microbial degradation in a screw cap centrifuge tube. The tube will be placed on a rotating device and mixed at 8-10 rpm for 6 h. The Tenax will be removed and extracted twice with hexane. The hexane extracts will be combined, analytical surrogates added and the volume reduced. The extracts will be analyzed by GC-ECD. Depending on the sediment concentration, it may be necessary to set up several tubes with Tenax resin and sediment in order to obtain sufficient mass of the desorbed contaminants for analysis. The desorption experiments will determine whether the duration of contact time between pesticide and particle affects desorbability (and potentially toxicity) when those particles are introduced into aquatic systems.

Facilities:

The facilities within the Fisheries and Illinois Aquaculture Center contains lab space equipped with fume hoods, environmental chambers, analytical scales, two Aiglent 6890 GCs equipped with nitrogen/phosphorous and electron capture detectors, an Aiglent 1100 HPLC, a Packard 1900TR liquid scintillation counter, and rearing facilities for the test organisms. Work with ^{14}C-labelled compounds is currently on going in Dr. Lydy's lab, and all regulatory approvals are in place. Similar bioassays and analytical methods have been performed at this facility, and we anticipate little difficulty in meeting the facilities needs of this project.

Dr. Donald Weston's components of the research will be conducted at the Richmond Field Station of the University of California, Berkeley. Dr. Weston's laboratory has been routinely used for toxicity testing of samples from California's Central Valley, as well as other sites. Cultures are maintained of all species that will be used in this project (H. azteca, C. tentans, L. variegatus). Temperature-controlled environmental chambers and recirculating baths are available for this project, as are microscopes, water quality instrumentation, analytical balances, and similar toxicity testing equipment.

The USGS Columbia Environmental Research Center (CERC) is located two miles southeast of Columbia, MO. The heart of the Center is a 25,000 square foot building with a spacious wet laboratory. Peripheral buildings provide additional space devoted to aquatic toxicology, environmental chemistry, fish culture, river studies, computer facilities, technical library service, and conference facilities. Two 1,100-foot deep wells provide an uncontaminated source of moderately hard water at a temperature of 17°C, suitable for maintaining both cold- and warm-water fishes and invertebrates. Research equipment includes large temperature controlled water baths used in toxicity testing, and flowing water test systems that are manipulated to simulate natural environmental conditions. These systems are designed to study long-term effects of environmental stressors on survival, growth, reproduction, physiology, and biochemistry of aquatic life under environmentally relevant conditions.

Related research:

Because of space limitations we have not provided a literature review of the current state of knowledge on pyrethroid fate and toxicology, however one study deserves discussion as it forms the basis for much of the work proposed and demonstrates the feasibility of the approach. Drs. Weston and Lydy, both participants on the proposed project, are currently measuring pyrethroid concentrations in sediments (primarily in the San Joaquin watershed), and conducting toxicity testing of these sediments using C. tentans and H. azteca. Most of the pyrethroids proposed for analysis in this study are being analyzed under the current study, and detection limits (1 ng/g) has been sufficient to quantify pyrethroids in 16 out of 17 sites tested. A method development paper has already been submitted to the Journal of Chromatography summarizing the analytical results of this project (You et al., in review). In addition, detection limits were about 2 orders-of-magnitude below acutely toxic concentrations, which preliminary data suggests are at least 100 ng/g. The majority of the sites, while having pyrethoids present at low concentrations, failed to show evidence of toxicity. Three sites had severe toxicity believed to be related to their pyrethroid levels: Del Puerto Creek (130 ng/g total pyrethroids) and 2 lettuce tailwater ponds (140-500 ng/g). One site (San Joaquin River at Vernalis) showed moderate sediment toxicity to both test species, but due to unknown causes.

The work above represented the first significant effort to obtain a geographically-broad view of pyrethroids in the San Joaquin River watershed. On the basis of its success, Drs. Weston and Lydy have recently been asked by the State of California to do additional pyrethroid analysis and toxicity testing in more northern areas (largely the Delta of the Sacramento and San Joaquin Rivers). Sampling for this study will extend from April to August, 2003, and will focus on agricultual drains that discharge irrigation return flows to surface waters. The PRISM work, if funded, will extend the sampling area even further north into the Sacramento River watershed.

All these studies, when completed and taken together, will provide an invaluable data set on pyrethroid concentrations in agricultural and urban water bodies of California's Central Valley. They cumulatively will include approximately 100 samples taken for pyrethroid and organochlorine residues and toxicity testing, over a distance extending for 400 km. This is all the more remarkable considering that as little as a year ago, there were almost no data on pyrethroid concentrations in sediments from an agricultural region that provides a large portion of this country's produce. Given the lack of data, our studies of simply measuring concentration and toxicity have been a logical first step. However, what is now needed are more detailed studies of pyrethroid toxicology in order to better understand the significance of the environmental levels we have seen. The USGS funding we seek will support only minimal work to measure environmental pyrethroid levels since those data are becoming available through other channel. However, it will allow us to examine biological uptake processes, bioavailability issues, and interactions among multiple pesticides so that we may make better use of the emerging environmental data.

Training potential:

There exists a tremendous opportunity to train young scientists in environmental toxicology through this project. Most of the work will be conducted by two post-doctoral researchers, a graduate student, and 1-2 undergraduate students. These personnel will gain

experience in conducting sediment bioassays, taxonomic identification, learn various extraction and analytical techniques to identify and quantify pesticides from sediments and invertebrate tissues, and learn how to process data from the project into peer-reviewed publication.

Statement of Government Involvement:

This proposal represents a multi-institutional and multi-state effort designed to address an important regional/national problem. This collaboration between Dr. Donald Weston at the University of California, Berkeley, Dr. Michael Lydy at Southern Illinois University-Carbondale and Dr. Christopher Ingersoll at the USGS facility in Columbia, Missouri should provide for excellent cross-fertilization among these research labs and cross-training for all participants.

Dr. Ingersoll has agreed to participate in this project by extending the toxicity testing to examine chronic endpoints. His lab will conduct chronic H. azteca tests on sediments with measurable pyrethroid levels, but which our acute testing would suggest show no environmental threat. He will also be involved in overall integration and analysis of the project data. He has worked with ASTM and EPA in developing standard methods for conducting toxicity and bioaccumulation tests with contaminated sediments, and brings valuable expertise in the development of chronic toxicity tests with amphipods to this project.

Information Transfer Plan:

The proposed research will address many questions including:

- whether pyrethroid insecticides found in sediments in are causing acute and/or chronic toxicity to important invertebrate species;
- if native species are accumulate measurable quantities of pyrethriods and organochlorine insecticides, suggesting the possibility of trophic transfer;
- whether bioavailability or desoprtion rates of pyrethroids change as the soils age over time;
- if biotransformation is affecting bioaccumulation potential of selected pyrethroids;
  - whether mixtures of pesticides are acting in an additive fashion; and
  - if environmentally realistic PBO concentrations are enhancing pyrethroid toxicity.

Because of the diversity of questions we will answer, our data will be of interest to a broad audience including pesticide producers, the scientific community particularly in the areas of contaminant fate and toxicology, and environmental management agencies with interests either in pesticide regulation/monitoring or contaminated sediment management in general. We expect that our results could have substantial impact on how pesticides in sediments are monitored, and are likely to enter into regulatory decisions on which pesticides may be used and how their application is to be managed. In California, and we suspect many other locations in the country, emerging urban and/or agricultural use of pyrethroids has resulted in intense interest by environmental managers in research results such as will be provided by this study.

To reach potential users, we will disseminate project results through several mechanisms:
Publication in the peer-reviewed literature - Publication in this format is the best approach to reaching the scientific community, environmental managers, and pesticide producers. Publication in the journal Environmental Toxicology and Chemistry is the preferred outlet. Not only are our results are quite appropriate to its subject matter, but it reaches most of the user groups we have identified. The PRISM funded portion of the work will result in 1-2 publications, and the additional USGS components would provide 2-3 more.
Publication in IEP newsletter - The Interagency Ecological Program (IEP) is comprised of nine state and federal agencies with management responsibilities in the Sacramento and San Joaquin Rivers and Delta. The IEP publishes a periodic newsletter which includes research articles, and publication of our data through this route should reach all relevant agency staff.
Presentation at national conferences - This approach is preferred for rapid dissemination of project results as it is faster than publication in peer-reviewed literature. The annual meeting of the Society for Environmental Toxicology and Chemistry may be a particularly good forum because its membership has broad representation from academia, government, and private industry. We anticipate at least two presentations on results during the duration of the study.
Presentations in local forums - The preferred approach to reach state resource managers with regulatory interests in San Francisco Bay and its tributaries will be to present project results at

meetings in the Bay area. Good forums for this include the State of the Estuary Conference, the CALFED Science Conference, or the annual IEP meeting.

Participation of the Sacramento River Watershed Program (SRWP), a lead player in the PRISM project, will greatly facilitate the dissemination of research results. The SRWP is a stakeholder group consisting of many parties with interest in water quality within the Sacramento River watershed. The SRWP and its component committees include diverse groups including agricultural interests, urban stormwater representatives, state and federal environmental managers, environmental consultants, and academic scientists. There will be many briefings on research progress to both the SRWP Board of Trustees and relevant subcommittees.

References

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USEPA 2000. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates, second edition, EPA/600/R-99/064, Washington, DC.

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Hydrological regimes, pond morphology, habitat use: predicting the impact of an emerging aquatic pathogen

Basic Information

Title:	Hydrological regimes, pond morphology, habitat use: predicting the impact of an emerging aquatic pathogen
Project Number:	2004CA90B
Start Date:	3/1/2004
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	44
Research Category:	None
Focus Category:	Invasive Species, Ecology, None
Descriptors:	None
Principal Investigators:	Cheryl Briggs, Cheryl Briggs

Publication

1. PUBLICATIONS FROM PRIOR PROJECTS. Please provide citations to publications attributable to projects completed in prior years and not included in this year's annual report. Rachowicz, L. J., and V. T. Vredenburg. 2004. Transmission of Batrachochytrium dendrobatidis within and between amphibian life stages, Diseases of Aquatic Organisms, 61, 75-83. Rachowicz, L. J., J.-M. Hero, R. A. Alford, J. W. Taylor, J. A. T. Morgan, V. T. Vredenburg, J. P. Collins, and C. J. Briggs. (In Press) The novel and endemic pathogen hypotheses: competing explanations for the origin of emerging infectious diseases of wildlife, Conservation Biology. PUBLICATIONS & CITATION FORMAT: List all reports, in the following format, published during the reporting period resulting from work supported by your project funding and by supplemental grants during the reporting period. Briggs, C.J, Vredenburg, V.T., Knapp, R.A., and L. J. Rachowicz. (In Press) Investigating the population-level effects of chytridiomycosis, an emerging infectious disease of amphibians, Ecology. Rachowicz, L. J., R. A. Knapp, J. A. T. Morgan, M. J. Stice, V. T. Vredenburg, J. M. Parker, and C. J. Briggs (In Review) Emerging infectious disease as a proximate cause of amphibian mass mortality in Rana muscosa populations, Submitted to Ecology. Rachowicz, L. J., 2005, Transmission of an emerging infectious disease in a declining amphibian species: Batrachochytrium dendrobatidis in the mountain yellow-legged frog (Rana muscosa) " Ph.D. Dissertation," Department of Integrative Biology, University of California, Berkeley, California, 210 pages.

RESEARCH PROGRAM:

Project Summary

Declines in amphibian populations have been reported throughout the world in recent years. A number of factors have contributed to these population declines, including disease, introduce species, habitat loss and alteration, and climate change. Chytridiomycosis is a potentially fatal disease of amphibians caused by the chytrid fungus *Batrachochytrium dendrobatidis*, which has appeared recently in the aquatic habitats of California and throughout the world. In portions of the Sierra Nevada mountains of California, the disease is causing rapid die-offs of mountain yellow-legged frogs, *Rana muscosa*, a threatened native frog species. In other areas of the Sierra, infected populations of *R. muscosa* appear to be persisting with the fungus. In this study we are investigating why the fungal pathogen is having different outcomes on frog populations in different California watersheds.

The main hypothesis that we are investigating is that differences in the pond morphology and topography of the landscape in different areas result in the frogs using the habitat differently at the different sites, altering their risk of acquiring and succumbing to the disease. Frogs that spend most of their time aggregated in the main lakes and ponds at each site, and in colder temperature habitats, are at greater risk from the disease. Frog die-offs due to the disease in the Sierra are occurring mainly in areas consisting of deep lakes surrounded by granite bedrock, where the adult frogs spend the majority of their time in the lakes. The sites at which the frogs are persisting with the fungus include extensive marsh and stream areas with emergent vegetation, in addition to lakes. At these sites the frogs are not confined to isolated lakes, and may be able to escape from areas with high concentrations of zoospores (the infectious stage of the fungus by which the disease is spread). We are also investigating alternative hypotheses that differences in the transmission, infectivity, and/or virulence of the fungal strains, or differences in susceptibility of the frog genotypes at the different types of sites, are leading to the observed differences in the impact of the fungal pathogen.

Field Surveys: During the summer of 2004 we conducted detailed surveys at sites in the Sierra Nevada mountains experiencing *R. muscosa* die-offs due to chytridiomycosis, and sites with *B. dendrobatidis* present with persistent *R. muscosa* populations. We used a newly-developed real-time PCR (quantitative PCR) protocol to determine the infection status (presence/absence of *B. dendrobatidis*) and infection level (fungal loads) of *R. muscosa* individuals. This protocol involves non-destructive swabbing of the frog skin, and therefore can be used repeatedly on the same frog individuals. We performed repeated surveys at the field site over the course of summer 2004. At each site we quantified the abundance and stage-structure frog populations, the infection status of tadpoles and adults, documented the presence and abundance of other potential hosts for the disease, and quantified the habitat characteristics. At sites where marking was feasible (i.e. frog population densities were not too high) we marked adult *R. muscosa* using PIT tags (passive integrated transponder), and recaptured and re-swabbed the same individuals over the course of the summer. We found that infected frogs at die-off sites carried very high fungal loads, while at persistent sites the infected frogs were experiencing only low to moderate infections. Interestingly, at the persistent infected sites, some adult *R. muscosa* were found to lose the infection between the start and end of the summer. In the high Sierra, *R. muscosa* have a

very short summer season in which they are active, with ice-free days spanning from mid-June through late September. A great deal of mortality in *R. muscosa* occurs during the long overwintering period. Therefore, we will be repeating these surveys in the summer of 2005 to determine if infected *R. muscosa* individuals at the persistent sites are able to survive over the winter.

Laboratory Experiments: From winter through summer 2004 we conducted a laboratory experiment to investigate the ecological differences in fungal pathogen strains collected from infected frogs in die-off and persistent sites. We experimentally inoculated uninfected tadpoles that had been raised from eggs in the laboratory with a known amount of zoospores of a fungal strain from either a die-off site or a persistent site. Each week we inspected the tadpoles for signs of infection (to determine differences in transmission of the different strains), and measured the rate of release of infectious zoospores (to determine differences in infectivity of infected tadpoles). We followed the tadpoles through metamorphosis to record differences in survival of the individuals exposed to the different strains. In the experiment we found that transmission of the fungus to tadpoles requires very high doses of zoospores, but we measured no differences between the fungal strains in transmission, virulence, infectivity, or tadpole survival. Thus, so far we have found no evidence that differences in fungal strains are responsible for the different population-level impacts of the disease at the different sites. We are in the process of repeating this experiment using post-metamorphic *R. muscosa,* which are more susceptible than tadpoles to *B. dendrobatidis*.

Antimicrobial peptides released from the skin of frogs are part of their innate immune response, and some peptides have been shown to be effective at killing *B. dendrobatidis* in laboratory cultures. From fall 2004 through spring 2005, we conducted an experiment to determine if the antimicrobial peptides that are released from *R. muscosa* are effective in protecting *R. muscosa* from *B. dendrobatidis*. Differences in the antimicrobial peptide response might help explain the different outcomes of the fungal pathogen observed in different parts of the Sierra Nevada mountains. Through this experiment we also quantified the dose-response curve of *R. muscosa* to *B. dendrobatidis*. *R. muscosa* subadults were exposed to specific quantified doses of *B. dendrobatidis* zoospores, and monitored to determine the fraction that became infected, and the fate of the infected individuals. In some treatments, the antimicrobial peptides were removed from the frogs prior to exposure to *B. dendrobatidis*. We found that *R. muscosa* individuals increased their peptide production following exposure to *B. dendrobatidis*. It was predicted that if antimicrobial peptides served to defend the frogs against the disease, then individuals from which peptides had been removed prior to exposure would be more likely to become infected. However, we found absolutely no difference between the fractions of individuals that became after peptide removal versus those that were exposed to the same dose without prior peptide removal. This suggested that the antimicrobial peptides are not sufficient to protect *R. muscosa* from chytridiomycosis in even a simple experimental situation.

INFORMATION TRANSFER PROGRAM:

None

STUDENT SUPPORT:

Undergraduate student Eleanore Sternberg was supported in part on the Water resources grant.

Graduate students: Lara Rachowicz and Mary Stice were each supported for part of the year on the Water Resources grant.

	Total Project Funding		Supplemental Awards	Total
	Federal Funding	State Funding		
Undergrad.	0.5	0.5	0	1
Masters	0	0	0	0
PhD.	1	1	0	2
Post-Doc.	0	0	0	0
Total	1.5	1.5	0	3

NOTABLE ACHIEVEMENTS AND AWARDS:

No awards. Notable achievements include the key results described above.

PUBLICATIONS FROM PRIOR PROJECTS. Please provide citations to publications attributable to projects completed in prior years and not included in this year's annual report.

Rachowicz, L. J., and V. T. Vredenburg. 2004. Transmission of *Batrachochytrium dendrobatidis* within and between amphibian life stages, Diseases of Aquatic Organisms, 61, 75-83.

Rachowicz, L. J., J.-M. Hero, R. A. Alford, J. W. Taylor, J. A. T. Morgan, V. T. Vredenburg, J. P. Collins, and C. J. Briggs. (In Press) The novel and endemic pathogen hypotheses: competing explanations for the origin of emerging infectious diseases of wildlife, Conservation Biology.

PUBLICATIONS & CITATION FORMAT: List all reports, in the following format, published during the reporting period resulting from work supported by your project funding and by supplemental grants during the reporting period.

Briggs, C.J, Vredenburg, V.T., Knapp, R.A., and L. J. Rachowicz. (In Press) Investigating the population-level effects of chytridiomycosis, an emerging infectious disease of amphibians, Ecology.

Rachowicz, L. J., R. A. Knapp, J. A. T. Morgan, M. J. Stice, V. T. Vredenburg, J. M. Parker, and C. J. Briggs (In Review) Emerging infectious disease as a proximate cause of amphibian mass mortality in *Rana muscosa* populations, Submitted to Ecology.

Rachowicz, L. J., 2005, Transmission of an emerging infectious disease in a declining amphibian species: *Batrachochytrium dendrobatidis* in the mountain yellow-legged frog (*Rana muscosa*) " Ph.D. Dissertation," Department of Integrative Biology, University of California, Berkeley, California, 210 pages.

Using Marine Derived Nitrogen in Tree Rings to Assess Nutrient Flux and Salmon Escapement

Basic Information

Title:	Using Marine Derived Nitrogen in Tree Rings to Assess Nutrient Flux and Salmon Escapement
Project Number:	2004CA91B
Start Date:	3/1/2004
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	44
Research Category:	None
Focus Category:	Nutrients, Ecology, None
Descriptors:	None
Principal Investigators:	Michael Johnson, Michael Johnson

Publication

RESEARCH PROGRAM:

Problem Statement:

Pacific salmon (*Oncorhynchus* spp.) have disappeared from approximately 40% of their historic range in Washington, Oregon, Idaho, and California. Declining salmon populations represent much more than just a cultural and economic loss as these fish play a key role in the ecological integrity of many rivers and their watersheds. Adult salmon migrating to freshwaters to spawn transport substantial quantities of biologically important marine-derived nutrients (MDN) in their body tissues. The decomposition of post-spawning carcasses liberates these nutrients into their natal watersheds where they are utilized by aquatic and terrestrial plants and animals. Overwhelming recent evidence from streams and rivers along the Pacific coast suggests that MDN vectored by spawning salmon improve aquatic and terrestrial productivity in their natal watersheds and help enhance the growth and survivorship of future generations of salmon.

Successful conservation of anadromous salmon populations, and restoration of many freshwater ecosystems, will likely require mitigation for the loss of marine nutrients to spawning and rearing habitats. Current fisheries management, however, is hindered by a scarcity of data regarding historical escapement (i.e., number of fish that escape the ocean fishery and return to freshwater to spawn) to individual watersheds. Trends for individual watersheds are largely based on anecdotal information and provide little basis for estimating the true extent of the decline.

Research objectives:

Our research aims to develop a novel method to reconstruct historic salmonid escapement at the scale of individual watersheds. This objective is achieved through chemical analysis of naturally abundant stable isotopes in riparian trees growing adjacent to salmon streams. A fortunate product of feeding in the marine environment is that adult anadromous salmon are uniquely enriched with heavier isotopic forms of many elements (e.g., N, C, and S) relative to terrestrial or freshwater sources of these same elements. Recent advances in stable isotope analytical techniques allow the quantification of marine-derived nitrogen (MD-nitrogen) in annual growth rings of riparian trees. This innovation makes it possible to assess yearly variation in the uptake of this important nitrogen source over the life of the tree. Annual growth rings formed by a tree represent an integration of the environmental conditions under which it grew and recent research suggests that levels of MD-nitrogen in annual tree rings are directly related to densities of salmon in adjacent spawning reaches. We use non-destructive increment core (tree ring) samples collected from live riparian trees to quantify annual sequestration and incorporation of MD-nitrogen. Yearly nitrogen isotope levels are correlated to known annual salmon escapement data, tree growth, and stream proximity. The resulting relationships are then used to model historic salmon returns for periods where no such information exists and provide otherwise unobtainable data regarding watershed-specific population trends. Our research has three specific objectives:

1) Determine if there is a relationship between $\delta^{15}N$ in annual rings of riparian trees and historic salmonid escapement.

2) Determine if tree growth and $\delta^{15}N$ are related to the number of fish returning to spawn in the year the ring was formed or if growth is a product of accumulated nitrogen from previous years; i.e., is there a time lag present between numbers of salmon spawning and the growth and $\delta^{15}N$ content of the tree rings.
3) Determine if $\delta^{15}N$ in annual tree rings decreases as a function of distance from the active stream channel.

Methodology:

To examine the relationship between annual salmon escapement and tree ring nitrogen levels we have initiated research in three northern California watersheds: Mill Creek, a tributary of the Smith River; the Salmon River, a tributary of the Klamath River; and Waddell Creek, a small coastal watershed in Santa Cruz County. In each study watershed we established a series of 8 transects that run perpendicular to the active stream channel. Four transects were adjacent to active spawning reaches (salmon sites) and 4 transects were adjacent to reaches located upstream of impoundments that block migrating fish (reference sites). Along each transect, we sampled the nearest conifer tree at distances of 5, 10, 25, 50 and 100 from the stream. For each tree sampled we measured diameter at breast height, distance from the stream channel, canopy cover, and transect slope. Additionally, paired increment cores were collected using manual increment borers. A large diameter (12.0 mm) increment core was used to determine total N and stable nitrogen isotope ratios. The second core sample (5.15 mm diameter) was extracted and prepared for dendrological analysis using standard techniques.

In the laboratory, small-diameter increment cores were dried, permanently mounted, and sanded for analysis of annual growth rings. Prepared cores were converted to digital images and ring widths measured using an image analysis system. Annual growth from large diameter cores was excised using a razor blade and dissecting microscope at 10X magnification. Individual wood samples were transferred to stainless steel capsules and ground to a fine powder in high-speed amalgamator. Samples of ~30 mg of powdered wood were weighed into 8 x 5 mm tin capsules for stable nitrogen isotope analysis ($\delta^{15}N$).

Principle Findings:

Our results to date demonstrate that riparian trees growing adjacent to salmon-bearing streams are significantly enriched in salmon-derived nitrogen ($\delta^{15}N$) relative to control sites and the importance of this nitrogen source generally attenuates with increasing distance from the stream. Moreover, annual tree growth, percent nitrogen content of the wood, and $\delta^{15}N$ are all positively correlated with the number of salmon returning to spawn in previous years (a time lag exists between salmon-derived nitrogen delivery and tree response). We used quantitative relationships between annual tree ring variables ($\delta^{15}N$, % N, and growth) and the number of spawning salmon (escapement) to create a predictive model specifically for the Mill Creek watershed. The overall efficacy of our model was validated for years of known salmon returns (1980-2003) and used to reconstruct returns for the period 1946-1979 (23 additional years). We are currently processing additional samples from the Mill Creek site that will enable us to further extend our reconstruction and provide many additional years of previously unobtainable

salmon abundance information. Moreover, we are developing similar models for the Salmon River and Waddell Creek watersheds.

Significance:

The ability to determine current and historic contributions of MD-nitrogen to riparian trees provides a novel opportunity to assess the transfer of marine nutrients into riparian and freshwater ecosystems. Long-lived riparian tree species serve as valuable records of past biological events such as salmon declines or extirpations. Our preliminary research has determined that secondary and tertiary-growth trees routinely allow us to build chronologies that extend back more than 80 years, while old-growth forests provide centuries of historic data. Once predictable relationships between tree ring nitrogen levels and salmonid escapement are derived, it then becomes possible to reconstruct historic salmon returns for watersheds where old-growth trees still exist and escapement records do not. Nearly all recovery programs are built upon very uncertain estimates of population sizes prior to European settlement. Robust determination of such information would greatly assist resource managers in identifying and establishing appropriate restoration targets.

1. Provide publication citations associated with the research project.

No publications to date.

INFORMATION TRANSFER PROGRAM

This project is not designed as an information transfer project.

2. Provide publication citations associated with the information transfer project when applicable.

No publications to date.

STUDENT SUPPORT

	Total Project Funding		Supplemental Awards	Total
	Federal Funding	State Funding		
Undergrad.				
Masters				
PhD.	$14,276	$14,224		
Post-Doc.				
Total	$14,276	$14,224		$28,500

NOTABLE ACHIEVEMENTS AND AWARDS

None to report.

PUBLICATIONS FROM PRIOR PROJECTS

None to report.

Feasibility of Snowpack Characterization Using Remote Sensing and Advanced Data Assimilation Techniques

Basic Information

Title:	Feasibility of Snowpack Characterization Using Remote Sensing and Advanced Data Assimilation Techniques
Project Number:	2004CA92B
Start Date:	3/1/2004
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	44
Research Category:	None
Focus Category:	Climatological Processes, Methods, None
Descriptors:	None
Principal Investigators:	Steven Margulis

Publication

1. 1. Articles in Refereed Scientific Journals Durand, Michael, and Margulis, Steven, 2005: Feasibility test of multi-frequency radiometric data assimilation to estimate snow water equivalent, Journal of Hydrometeorology, accepted. 6. Other Publications Durand, M. and S.A. Margulis, 2005: Large-scale SWE Estimation: Optimal Use of Remote Sensing and Snow Modeling, Southwest Hydrology, 4(2), 20-21,32.

RESEARCH PROGRAM:

Many semi-arid regions of the world, including California, depend on annual snowmelt for the majority of their water supply. However the primary method for estimating the amount of water stored in the snow pack (snow water equivalent, SWE) is still done by field snow surveys. This approach is extremely limited because the survey data are sparse point estimates and because they rely upon regression and comparison to historical measurements.

New methods for estimation of SWE have been developed recently. For the past several decades, inversion of remote sensing data and application of snow models have been used to estimate SWE. However SWE estimates based solely on either remote sensing inversion or snowpack modeling techniques are contain large uncertainty. For retrieval methods, the uncertainty lies primarily in the relationship between the snow states and the remote sensing observations. For modeling, errors occur primarily as a result of the propagation of uncertainty in model inputs (e.g., precipitation) to the SWE estimates. It is this uncertainty that motivates the development of the data assimilation approach used in this project.

Data assimilation methods, such as the Ensemble Kalman Filter (EnKF) are used to merge remote sensing observations into a hydrologic model to produce spatially distributed estimates of SWE over the entire basin. The EnKF weighs the relative uncertainty of the model and of the observations and provides an estimate of the state variable as well as an estimate of its uncertainty. This project constitutes a feasibility study for estimating SWE through the incorporation of remote sensing observations in the microwave, visible, and thermal infrared parts of the spectrum into a physically-based snow model.

The first phase of our work focused on the selection and development of the required snow and remote sensing models needed in the data assimilation approach. We have chosen two widely accepted models as the foundation of the data assimilation framework. During the preliminary testing phase we have incorporated more realistic models for the snow grain diameter evolution and snow albedo (reflectivity) which were found to be extremely important parameters in the radiative transfer model. These model refinements should ultimately significantly improve the SWE estimates. We have applied the model to data in the Mammoth Mountain region in the Sierra Nevada. These models have been embedded in a data assimilation framework (EnKF) to the ability to estimate SWE from remotely sensed microwave radiobrightness observations.

As a first step, synthetic experiments at the point-scale were performed to test the feasibility of the approach. In these tests, synthetic realizations of the remotely sensed observations were used in the assimilation scheme to test whether the true snowpack characteristics could be recovered under conditions of uncertain initial conditions and precipitation. The methodology was shown to outperform commonly used retrieval methods and overcome significant biases often seen in precipitation in mountainous regions. This part of the study has been recently accepted for publication (see reference below). Our future work will extend the method to a spatially distributed basin-scale application.

INFORMATION TRANSFER PROGRAM:

No information transfer activities to report.

STUDENT SUPPORT:

Michael Durand, graduate, Ph.D.; Civil and Environmental Engineering, UC Los Angeles

NOTABLE ACHIEVEMENTS AND AWARDS:

The preliminary work started in this project was helpful in obtaining a NASA New Investigator Program (NIP) award. This supplemental funding will allow for extension to the work after completion of the project.

PUBLICATIONS:

1. Articles in Refereed Scientific Journals
Durand, Michael, and Margulis, Steven, 2005: Feasibility test of multi-frequency radiometric data assimilation to estimate snow water equivalent, Journal of Hydrometeorology, accepted.
2. Book Chapter
None.
3. Dissertations
None.
4. Water Resources Research Institute Reports
None.
5. Conference Proceedings
None.
6. Other Publications
Durand, M. and S.A. Margulis, 2005: Large-scale SWE Estimation: Optimal Use of Remote Sensing and Snow Modeling, Southwest Hydrology, 4(2), 20-21,32.

Modeling and Optimization of Seawater Intrusion Barriers in Southern California Coastal Plain

Basic Information

Title:	Modeling and Optimization of Seawater Intrusion Barriers in Southern California Coastal Plain
Project Number:	2004CA93B
Start Date:	3/1/2004
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	44
Research Category:	None
Focus Category:	Groundwater, Management and Planning, None
Descriptors:	None
Principal Investigators:	William W-G. Yeh

Publication

RESEARCH PROPRAM

Project summary:

Seawater (saltwater) intrusion is a problem that threatens many coastal aquifers around the world. The problem may occur for many reasons but is generally a result of over-pumping of groundwater in the coastal area. An effective way of mitigating seawater intrusion has been the use of hydraulic barriers, or simply barriers. A barrier is essentially an array of injection wells arranged parallel and in close proximity to the coastline. The wells inject freshwater into the lower aquifers to raise the water level and to create a hydraulic barrier to stop seawater intrusion and to protect freshwater pumping wells in the coastal plain. Today, there are three major barriers in operation in Los Angeles County which protect a 20,300,000 acre-foot groundwater reservoir that is used to meet approximately 35% of the potable water supply for 3.2 million residents.

Though the barriers in LA County have been in operation since 1950's, there exist no systematic procedures to guide the operation. Recently, various deficiencies have been noticed in their performance and some regions of the aquifers have suffered leakage. This leakage of seawater through the barrier has degraded the groundwater basin water quality, reduced the net groundwater basin storage, caused shutdowns of freshwater pumping wells, and caused significant losses in basin management activities. Furthermore, a significant loss of injected water (20 percent) has been identified due to seaward migration of the injected water. These deficiencies can be mitigated by optimizing the operation of the barrier facilities or, if necessary, constructing additional injection wells, or both.

The goal of this research is to use state-of-the-art groundwater modeling and optimization techniques to develop optimal management strategies for the Alamitos barrier, one of the three barriers operated by the LA County Department of Public Works. Specifically the objectives are: (1) to first calibrate and validate a groundwater model to simulate the complex barrier operations, (2) to determine the optimal management strategy of the existing barrier facilities, (3) to identify the optimal candidate sites for additional injection wells, and (4) to use multiobjective optimization to investigate alternative and competing management strategies that may be cost effective in addressing the seawater intrusion problem.

Currently the calibration has been completed and documentation has been ongoing during the transition to the management phase. Two alternative methods were applied to calibrate flow parameters: the classic linked-simulation-optimization (LSO) and the newly developed natural-neighbor-kriging (NNK). NNK proved to have a number of advantages over the classic LSO method specifically, computational efficiency (results are obtained in minutes rather than days for the LSO), reproducibility of results (solution is not predicated on an initial guess), and a debatable improvement in the reliability of parameterization results. In fact, estimating a comparable degree of parameter complexity by classical means was simply infeasible in terms of computational requirements. Furthermore, the NNK results were shown to be roughly consistent with a previous geologic study of the site. The primary disadvantage of NNK is shared by most inverse methods, which is that the accuracy of the results is fundamentally based on a set of field measurements.

A number of LSO simulation runs confirmed general consensus in this field that the optimal solution is highly dependent on the initial basis, and the problem suffers from a non-uniqueness in minimization of a highly nonlinear least-squares objective. Transport calibration required significantly longer simulation runs to maintain accuracy. An optimal homogeneous transport parameter set was identified as the model error could not be reduced further by increasing the transport parameter complexity. A draft manuscript has been completed describing the model

calibration phase and is expected to be formally submitted for publication in Summer 2005. Completion of this study is slated for June 2006.

Publications:

Not available

Professional Presentations:

Bray, Ben S, Youn Sim, William W-G. Yeh. 2004. "Calibration of a Complex Three-Dimensional Coastal Aquifer with Density-Dependent Flow." Poster Presented at American Geophysical Union 2004 Fall Meeting, San Francisco, Dec. 13-17, 2004. ID: H33F-0520.
Note: Abstract available online: <http://www.agu.org/meetings/fm04/fm04-sessions/fm04_H33F.html >
Last accessed: 6-14-05

STUDENT SUPPORT

During the reporting period (March 1, 2004 to February 29, 2005), funds were used to support PhD student, Ben Bray. Ben has successfully passed his PhD qualifying exam and is expected to complete his PhD degree in June 2006.

	Total Project Funding		Supplemental Awards	Total
	Federal Funding	State Funding		
Undergrad.				
Masters				
PhD.	$16,367.86			
Post-Doc.				
Total	$16,367.86			

NOTABLE ACHIEVEMENTS AND AWARDS:

Not applicable

PUBLICATIONS FROM PRIOR PROJECTS:

Tu, M-Y, F. T-C. Tsai and W. W-G. Yeh, "Optimization of Water Distribution and Water Quality by Hybrid Genetic Algorithm," to appear in *Journal of Water Resources Planning and Management,* ASCE, 2005.

Development of a Quantitative Detection Method for Enumerating Host-Specific Fecal Bacteria Based on Real-Time, Quantitative Polymerase Chain Reaction

Basic Information

Title:	Development of a Quantitative Detection Method for Enumerating Host-Specific Fecal Bacteria Based on Real-Time, Quantitative Polymerase Chain Reaction
Project Number:	2004CA94B
Start Date:	3/1/2004
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	44
Research Category:	None
Focus Category:	Methods, Water Quality, None
Descriptors:	None
Principal Investigators:	Kara Nelson

Publication

RESEARCH PROGRAM: Updated project information. The process for submitting each research project consists of the following progressive steps:

1. Include Problem and Research Objectives, Methodology and Principal Findings and Significance for your project.

   Problem and Research Objectives: The goal of the proposed research is to develop and evaluate a quantitative method for calculating the fractional contribution of fecal pollution from human and animal sources by measuring host-specific fecal indicator bacteria using the real-time, quantitative polymerase chain reaction (QPCR).

   Methodology: The experimental methods include both laboratory and field research. In the laboratory, we are developing QPCR methods for total *E. coli* as well as several host-specific target sequences in fecal indicator bacteria (other than *E. coli*). In the field, we are characterizing the sources of fecal pollution in a watershed by combining our QPCR methods with traditional water quality measurements, such as culturable *E. coli*, nutrients, and BOD.

   Principal Findings: The first activity was focused on developing a QPCR method for total *E. coli*. The main challenge was to eliminate trace levels of contamination in commercial preparations of the polymerase enzyme, which interfere with detection of low levels of *E. coli*. A novel DNase treatment step was developed that may have widespread relevance for many QPCR applications. This phase is near completion and a manuscript is in preparation for submission to a peer-reviewed journal. The next activity is to develop QPCR methods for several host-specific targets that we have identified from the literature and discussions with colleagues.

   Significance: Fecal pollution continues to be among the leading contaminants of our nation's waters. This research will result in improved tools to manage fecal pollution, ultimately increasing our ability to identify and target the dominant sources of pollution, to monitor changes in the concentration of fecal pollution and its sources over time, to assess the effectiveness of specific mitigation strategies, and to provide more information for evaluating the true public health risks.

2. Provide publication citations associated with the research project.

   none to date

3. You have the option of providing introductory text regarding your overall research program.

   Kara Nelson received her PhD in Environmental Engineering from the University of California, Davis in 2001. Since 2001, she has been an assistant professor in the Dept. of Civil and Environmental Engineering at the University of California, Berkeley. Prof. Nelson teaches classes in environmental science, physical-chemical treatment processes and the control of waterborne pathogens. Her research areas include natural systems for water and wastewater treatment, the detection and inactivation of pathogens in water and sludge, and appropriate technologies for improving water quality in developing countries.

INFORMATION TRANSFER PROGRAM: Provide a brief description of information transfer activities supported with section 104 and required matching funds during the reporting period.

Not applicable.

STUDENT SUPPORT: A summary of the number of students supported resulting from work supported by your project funding and by supplemental grants during the reporting period.

None of the federal project funds were spent on student support during the reporting period; however, the funds will be spent on student support during the next reporting period.

	Total Project Funding		Supplemental Awards	Total
	Federal Funding	State Funding		
Undergrad.				
Masters				
PhD.		20,009.85		20,009.85
Post-Doc.				
Total				20,009.85

NOTABLE ACHIEVEMENTS AND AWARDS: Provide a brief description of any especially notable achievements and awards resulting from work supported by your project funding and by supplemental grants during the reporting period.

None to date.

PUBLICATIONS FROM PRIOR PROJECTS. Please provide citations to publications attributable to projects completed in prior years and not included in this year's annual report.
None to date.

PUBLICATIONS & CITATION FORMAT: List all reports, in the following format, published during the reporting period resulting from work supported by your project funding and by supplemental grants during the reporting period.

1. Articles in Refereed Scientific Journals
none to date (in preparation)
2. Book Chapter
3. Dissertations
none to date (in preparation)
4. Water Resources Research Institute Reports
5. Conference Proceedings
6. Other Publications

Institutional Re-arrangements: forging smart use water policy coalitions at the intersection of geo-technical engineering with urban open space

Basic Information

Title:	Institutional Re-arrangements: forging smart use water policy coalitions at the intersection of geo-technical engineering with urban open space
Project Number:	2004CA110G
Start Date:	9/1/2004
End Date:	7/31/2006
Funding Source:	104G
Congressional District:	48
Research Category:	Social Sciences
Focus Category:	Law, Institutions, and Policy, Hydrology, Recreation
Descriptors:	None
Principal Investigators:	Helen Ingram

Publication

1. No reports or articles were published during this period.

1) Research Synopsis

Between September 1, 2004 and February 28, 2005 the following research activities took place:

- A media survey for the four case study cities was conducted. Based on newspaper sources, publicly available meeting minutes and agency websites, and published sources (manuscripts, journal articles, books) treating local development, environmental and watershed histories, a preliminary list of interview subjects was compiled.
- Informal fact-finding was conducted over the telephone and e-mail to further characterize the coalition history and membership in each city.
- Four cities were finalized as study sites: Tempe, AZ; San Jose, CA; Denver, CO; and Los Angeles, CA.
- A preliminary map of coalition actors and their institutional affiliations was created for each site.
- A semi-structured interview questionnaire was developed. Drawn from the theoretical tenets of social movement theory, policy studies, and urban development, twenty open-ended questions were drafted and finalized as the basis for interview research.
- Site visits and participant observation commenced in two cities: Tempe (February 15-17) and Los Angeles (January 5, 19, 24; February 12). Data from each visit was noted and transcribed.
- Preliminary codes and analytic memos were developed for the initial data set (data collection continued into the spring, and will proceed through the fall).

2) Publications

No reports or articles were published during this period.

3) Information transfer program

On January 22, 2005 Anne Wessells gave a presentation at the Greening XIV Conference at UC Irvine. Greening is an annual environmental policy conference sponsored by alternating Southern California universities. Her presentation, using the same title as the grant, acknowledged the support of NIWR/USGS and reviewed the theoretical framework and progress to date of the research project. The investigators received positive and helpful feedback from colleagues at the University of Southern California, Harvey Mudd College, UCLA, Cal State Northridge, and UC Riverside.

In February, a paper abstract based on this research was submitted and accepted under the "Environmental Management" track for the national conference of planning educators (ACSP). The paper will be presented at the ACSP conference in Kansas City, MO, October 27-30, 2005. NIWR/USGS support for the research was noted in the abstract submission.

4) Student Support

U.C.I. doctoral student Anne Wessells was fully supported throughout the reporting period. Her in-state student fees, as well as her monthly Graduate Student Researcher salary, have been made possible through this grant. Ms. Wessells was recently advanced to candidacy, and is making the governance challenges of urban watershed management the subject of her dissertation.

5) Student Internship Program

There were no student interns during this period.

6) Notable Achievements and Awards

The Principal Investigator, Helen Ingram, was invited to give a talk on related research issues to the National Academies of Science Sackler Symposium on Water, in October 2004. Her talk, "The Importance of Institutions and Policy in Resolving Global and Regional Water Scarcity," was presented at the Sackler Colloquium of the National Academy of Sciences, The Role of Science in Solving the Earth's Emerging Water Problems, Beckman Center of the National Academies, Irvine, California, October 8-10, 2004.
Dr. Ingram was also named a Member of the Board of Electors for the James Martin Professorship of Technology and Social Change, Oxford University, United Kingdom, in January 2005.

Information Transfer Program

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 RCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	.5	0	0	.5	0
Masters	0	0	0	0	0
Ph.D.	4	1	0	5	10
Post-Doc.	0	0	0	0	0
Total	4	1	0	5	10

Notable Awards and Achievements

The preliminary work started in this project was helpful in obtaining a NASA New Investigator Program (NIP) award. This supplemental funding will allow for extension to the work after completion of the project.

The Principal Investigator, Helen Ingram, was invited to give a talk on related research issues to the National Academies of Science Sackler Symposium on Water, in October 2004. Her talk, The Importance of Institutions and Policy in Resolving Global and Regional Water Scarcity, was presented at the Sackler Colloquium of the National Academy of Sciences, The Role of Science in Solving the Earths Emerging Water Problems, Beckman Center of the National Academies, Irvine, California, October 8-10, 2004. Dr. Ingram was also named a Member of the Board of Electors for the James Martin Professorship of Technology and Social Change, Oxford University, United Kingdom, in January 2005.

Bray, Ben S, Youn Sim, William W-G. Yeh. 2004. Calibration of a Complex Three-Dimensional Coastal Aquifer with Density-Dependent Flow. Poster Presented at American Geophysical Union 2004 Fall Meeting, San Francisco, Dec. 13-17, 2004. ID: H33F-0520. Note: Abstract available online: Last accessed: 6-14-05

Publications from Prior Projects