Water Resources Center
Annual Technical Report
FY 2003

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 7 new projects and continued 16 projects for a total of $629,614.00 with nearly every UC Campus participating.

Structure and seasonal changes of nematode communities from vernal pools (Santa Rosa Plateau)

Basic Information

Title:	Structure and seasonal changes of nematode communities from vernal pools (Santa Rosa Plateau)
Project Number:	2003CA35B
Start Date:	3/1/2002
End Date:	2/28/2003
Funding Source:	104B
Congressional District:	44
Research Category:	Not Applicable
Focus Category:	Ecology, Wetlands, None
Descriptors:	
Principal Investigators:	Paul De Ley

Publication

Structure and seasonal changes of nematode communities from vernal pools (Santa Rosa Plateau)

Introduction

Nematodes are diverse and abundant in soils and sediments, occupying a wide range of ecological roles that reflects the overall condition of the microbiological ecosystem. Our project constitutes the first study of nematode communities from vernal pools. It aims to provide the first ecological and taxonomic data from this fragile and biologically important habitat, through a combined morphological and molecular survey of two pools in the Santa Rosa Plateau Ecological Reserve (SRPER). These data will be analysed for the purposes of ecosystem health monitoring, and for possible occurrence of nematodes parasitizing the locally occurring endangered species of plants and fairy shrimp.

Research Program

During the first year of this project, our activities consisted mainly of the training of undergraduate student helpers, and the collection of two sample series. State permits were approved by October 2002, but unfortunately the federal permit took until May 16th 2003 to arrive. The first sample series was collected on June 11th 2003, each sample consisted of five 200 ml cores taken randomly with a 6x2 inch auger in between plant stems (to make sure no plants were removed), within a 1 m^2 square. Limited physicochemical measurements (temperature, pH, conductivity, oxidation reduction potential) were taken on-site. Soil texture and moisture were manually and visually assessed on-site.

Two samples were collected from each of two vernal pools during each sampling series. The four resulting samples were taken to the nematology greenhouse for extraction of fairy shrimp cysts and nematodes, using sieving and decanting methods. In the following days and weeks, nematodes were processed for video microscopy and PCR as outlined in the original proposal. The remainder from each sample was fixed in hot formalin-glycerin 4:1, transferred to pure glycerin and 100 randomly picked individuals were mounted in permanent slides for identification.

The second sample series was collected on November 3rd, 2003 and processed as before, with two additions: water contents was determined by druing a subsample, and visual inspection of the root and plant debris fraction was followed by overnight extraction in a mist chamber.

Genera and species identified to date

The table below is a list of all nematode taxa isolated between March 2003 and February 2004 from SRPER. Genus identifications are largely complete and have yielded no fewer than 51 different genera. Species identifications are in progress, nine species were

identified to date and three of these are potentially new to science (confirmation requires literature from various obscure sources that are still being tracked down).

Achromadora	*Mesorhabditis*
Acrobeloides	*Microdorylaimus*
Anaplectus sp. cf. *granulosus*	*Monhystrella*
Aphelenchoides	*Neopsilenchus*
Aphelenchus	Nordiidae
Aporcelaimellus	Nygolaimidae
Basiria	*Nygolaimus*
Boleodorus	*Panagrolaimus*
Cephalobidae	*Paractinolaimus* new. sp.??
Cephalobus new sp.??	*Paraphanolaimus*
Chromadoridae	*Plectus*
Coslenchus rhombus	*Pratylenchus*
Ditylenchus	*Prismatolaimus*
Dorylaimellus	*Prodesmodora*
Dorylaimus new. sp.??	*Psilenchus*
Enchodelus	Rhabditidae
Ethmolaimus	*Rhabdolaimus*
Eucephalobus	*Tobrilia imberbis*
Eudorylaimus	*Tobrilus nicasimilis*
Filenchus	Tylenchidae
Heteroderidae	*Tylencholaimellus*
Hirschmanniella pomponiensis	*Tylencholaimus*
Labronema	*Tylenchorhynchus*
Labronemella czernowitziensis	*Tylenchus*
Mesodorylaimus	*Tylocephalus*
	Ypsylonellus

The nematode communities found at SRPER constitute a mixture of species typical for grasslands and/or freshwater sediments. Surprisingly, many “aquatic” nematodes were still recovered in November, five months after the last significant rainfall and just a few days after some very light rain. These include large species (by nematode standards) like *Dorylaimus* and *Paractinolaimus*, which are not known to be tolerant of desiccation. Furthermore, their occurrence in samples of heavy clay would seem to contradict established correlations between average nematode body size and soil texture. This apparent contradiction was partly resolved by mist chamber extractions of the roots and plant debris collected in the November samples: most of these large nematodes appear to survive dry periods within or between this organic debris, rather than inside the soil itself.

Other interesting nematodes found include a species of *Hirschmanniella* and *Tobrilia*. The first was provisionally identified as *H. pomponiensis*, a species originally described from Pomponio State Beach (San Gregorio, CA). Nematodes in this genus are specialized

parasites of plants growing in wet to waterlogged soil. The genus *Tobrilia* is considered extremely rare, only two species are known and their original descriptions constitute the only published reports in the entire nematological literature to date.
As our extraction procedures were designed for small nematodes, the unexpected importance of roots and plant debris as a reservoir precludes accurate quantitative analysis with current methods as approved in our state and federal permits. We are therefore preparing a request for amendation to our procedures, to allow us to apply longer mist extractions to roots and larger plant debris. However, it is of concern to us that the US Fish and Wildlife Service's processing time for any permit-related matters exceeds eight months, even for minor matters such as adding the name of a new staff member. For the time being, we will continue our sampling and interpret the results only in qualitative respects.

Numbers of cysts extracted and returned to sampling site

Our state and federal permits require us to collect all fairy shrimp cysts recovered from our samples and return them to the respective sampling sites. The numbers of cysts recovered from our samples in 2003 are:

sampling date	6/12/03				11/3/03			
sampling site	L1	L2	C1	C2	L1	L2	C1	C2
intact cysts	2	2	2	0	26	1	11	1
broken cysts	2	0	0	0	13	0	2	0

The total numbers of fairy shrimp cysts encountered are very small compared to the overall cyst bank at either pool, which probably contains billions of cysts. The large differences between successive samples from the same sites is probably due to a combination of their patchy distribution and our increasing skills at recognizing them among other particles. Our methods do not allow us to distinguish between breakage caused by our processing, versus breakage due to natural causes (i.e. prior to sampling), but even in a worst case scenario we estimate to have caused mortality no greater than one third of the extracted cysts.

No obvious evidence of cyst puncturing or parasitism by nematodes was encountered so far, but based on our current experience we suspect different observation tools are required to discover such evidence. All cysts found in these two sampling series belonged to the genus *Branchinecta*, so presumably they are cysts of the vernal pool fairy shrimp (*B. lynchi*).

Preliminary conclusions

No nematodes were encountered from groups that are indicative of eutrophication, bacterial blooms or toxic pollutants. This suggests that the pools have so far not suffered from significant pollution that could be caused by e.g. runoff from nearby residential or agricultural areas. Some of the encountered large nematodes are known to be voracious predators, and it is possible that their diet includes fairy shrimp cysts. Biogeographic

records for these and related nematodes are very scarce in California, but it is quite likely that they are widespread and a natural component of freshwater ecosystems.

Among the plant parasites, *Hirschmanniella pomponiensis* is unlikely to represent a threat to any of the endangered plants, as this species may well be a true native of the Santa Rosa Plateau. The occurrence of the family Heteroderidae requires further study, as this family includes many of the most important economically damaging plant parasites. Fortunately, this also means that diagnostic DNA sequences are available for a rapidly increasing number of species in this family, and molecular identification is in progress of this and other species from SRPER.

Student support

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

Note: two undergraduate students (Mrs Sheila Esfahani and Mrs Melissa Yoder) were directly supported by project funding, each partly from state and partly from federal funding. Hence the total of 2 for the Undergraduate section above. Funding from other sources (UCR startup funds) was used to support project-related activities by one Post-Doctoral Researcher (Mrs. Irma Tandingan De Ley).

Pyrethroid Insecticides in Nursery Runoff: Transport and Impact on Aquatic Invertebrates

Basic Information

Title:	Pyrethroid Insecticides in Nursery Runoff: Transport and Impact on Aquatic Invertebrates
Project Number:	2003CA38B
Start Date:	7/1/2002
End Date:	6/30/2004
Funding Source:	104B
Congressional District:	44
Research Category:	None
Focus Category:	Water Quality, Wetlands, Irrigation
Descriptors:	None
Principal Investigators:	Jay Gan, Jay Gan

Publication

Pyrethroid insecticides in nursery runoff: transport and impact on aquatic invertebrates
– Research Category II, Aquatic Ecosystems

INTRODUCTION: Synthetic pyrethroids are insecticides widely used in both agricultural and urban settings. With the use of organophosphate and carbamate insecticides being restricted, the use of synthetic pyrethroids is expected to further increase. Synthetic pyrethroids have high toxicity to fish and aquatic invertebrates. Recent studies show that surface runoff can transport synthetic pyrethroids into surface streams and can cause potential toxic effects to aquatic organisms. The ecotoxicological effect of pyrethroids in a surface stream will closely depend on its bioavailability, as pyrethroids are known to adsorb strongly to sediment particles and dissolved organic matter. The interaction of phase distribution and aquatic toxicity of pyrethroids is poorly understood at present. This lack of understanding will prevent accurate risk assessment and adoption of scientifically sound regulatory criteria.

Nursery production is a multi-million dollar industry in California. To maintain plant vigor, pesticides and fertilizers are used heavily at nursery sites, and such uses are often coupled with intensive overhead sprinkler and drip irrigation. These processes lead to phenomenal runoffs and discharge of pesticides, nutrients, and sediments in the runoff. Because many nurseries are situated in urban environments, nursery runoffs can impose direct threats to water quality of urban watersheds (e.g., creeks) and the well being of exposed residents. Residues of synthetic pyrethroids were found in runoff from a number of nurseries in the southern California region. This finding poses as an urgent issue for the Regional Water Quality Control Boards, the nursery industries, the local governments, as well as the chemical manufacturers. Consequently, there is an urgent for management practices that may reduce pesticide runoff from nurseries.

RESEARCH PROGRAM:

The overall objective of this project is to evaluate the potential impact of pyrethroid insecticides in nursery runoff on affected water bodies of urban watersheds. Specific objectives are:

1). Characterize nursery runoffs by examining levels and makeup of suspended solids and DOM and the association of bifenthrin with these components, and correlate runoff profiles with time, seasons, and on-site activities.
2). Investigate influence of suspended solids and DOM on the bioavailability and aquatic toxicity of bifenthrin in runoff.
3) Understand the persistence and partitioning of bifenthrin in nursery-derived sediment, and predict the scale and duration of the impact of runoff-borne pyrethroids on receiving water bodies of urban watersheds.

In current monitoring studies, the whole effluent is extracted, from which the total chemical concentration is determined. As the total chemical concentration includes also the fraction that is adsorbed to suspended solids and dissolved organic matter, the measured concentration does not indicate the bioavailable concentration and will likely lead to overestimation in ecotoxicity. We developed a solid phase microextraction (SPME) method that offers selective detection of the dissolved concentration. We further used this method to evaluate phase distribution behavior of bifenthrin and permethrin in stream and runoff waters. In stream water, the majority of synthetic pyrethroids was associated with the suspended solids, and to a lesser extent, with dissolved organic matter (DOM). The freely dissolved phase contributed only 0.4-1.0%. In runoff effluents, the freely dissolved concentration was 10-27% of the overall concentration. The predominant partitioning into the adsorbed phases implies that the toxicity of SPs in surface water is reduced due to decreased bioavailability. This also suggests that monitoring protocols that do not selectively define the freely dissolved phase can lead to significant overestimation of toxicity or water quality impacts by SPs.

In close collaboration with nursery growers, we have carried out studies to understand the fate and distribution of bifenthrin and permethrin in nursery runoff, and to develop best management practices (BMPs)

to reduce their load in the runoff. The experimental site was a 100-acre commercial nursery located in southern California. The BMPs included optimized irrigation schemes, use of sediment traps/ponds, addition of polyacrylamide (PAM) into the effluent, and establishment of a vegetative strip. Monitoring data showed that the BMPs were highly effective in reducing the runoff of the synthetic pyrethroids. The level of bifenthrin or permethrin in the runoff flow was consistently reduced by > 92%. The mechanism for pesticide reduction was removal of suspended solids caused by the series of BMPs. These BMPs are inexpensive and of low maintenance, and therefore are feasible for implementation by other nursery growers, or at other runoff sites.

Use of bioassays to assess the water quality of wastewater treatment plants for the occurrence of estrogens and androgens

Basic Information

Title:	Use of bioassays to assess the water quality of wastewater treatment plants for the occurrence of estrogens and androgens
Project Number:	2003CA39B
Start Date:	3/1/2002
End Date:	2/28/2003
Funding Source:	104B
Congressional District:	44
Research Category:	None
Focus Category:	Water Quality, Solute Transport, Treatment
Descriptors:	None
Principal Investigators:	Daniel K. Schlenk

Publication

Use of bioassays to assess the water quality of wastewater treatment plants for the occurrence of estrogens and androgens –

INTRODUCTION

Over the past decade, there has been a global concern regarding the discharge of chemicals that have the potential of altering the endocrine system of aquatic organisms into waterways. Entering the aquatic environment via wastewater discharge and other point sources, a variety of compounds may bind to the estrogen receptor of resident biota and elicit responses in the animals similar to those when the organisms are exposed to the endogenous hormone: 17β-estradiol (E2). Receiving waters and effluent of sewage treatment plants contain these chemicals in concentrations that have demonstrated adverse effects on the normal physiology and endocrinology of the exposed organisms.

To detect estrogenicity of water, egg-yoke precursor proteins (vitellogenin-VTG) have been utilized as a whole animal indicator in male egg-laying animals. Its synthesis is under the direct control of circulating E2 in oviparous non-mammal vertebrates during egg production (Wahli *et al.* 1981). VTG synthesis is normally limited to reproductive females. Male and juvenile organisms possess the gene, which remains quiescent under normal conditions, however the gene can be easily expressed upon exposure to elevated levels of estrogens. VTG produced in the liver is eventually incorporated into oocytes to provide a source of metabolic energy for the developing embryo. Since the synthesis of VTG in fish is under the control of estradiol, the induction of VTG in male oviparous animals has been considered a valid biomarker for the exposure to estrogenic chemicals.

In vitro assays have primarily been used to evaluate the estrogenicity of water. *In vitro* assays include ligand binding assays, cell proliferation assays, recombinant receptor-receptor assays, and yeast-based screen assays (YES assays). The YES assay uses yeast cells containing the human estrogen receptor (ER), the estrogen-responsive element, and the *LacZ* gene as a reporter coding for β-galactosidase (Routledge and Sumpter 1996). In order for YES activation, an estrogen receptor ligand must penetrate the cell wall of the yeast and bind to the receptor. Compounds that do not bind the ER, but augment endogenous E2 concentrations would not be detected as environmental estrogens utilizing the YES assay.

RESEARCH PROGRAM

Numerous strategies have been implemented in an effort to ameliorate the adverse effects of insufficiently treated wastewater in eliminate endocrine-disrupting compounds. Two processes that have been commonly employed include enhanced wastewater treatment and wetland treatment. The objectives of this study was to use *in vivo* (rainbow trout VTG) and *in vitro* (yeast estrogen screening) assays to:

1) Investigate the estrogenic potencies of tertiary-treated wastewater, and
2) Evaluate the estrogenic activites of wastewater following wetland treatment

Methodology

Rainbow trout (*Oncorhynchus mykiss*) have been widely used to evaluate the effects of endocrine disrupting chemicals (Jobling *et al.* 1996; Thorpe *et al.* 2000; Ackermann *et al.* 2002; Schwaiger *et al.* 2002). Juvenile rainbow trout (Length:10.5 ± 1.2 cm) were provided by the California Department of Fish and Game Mojave River Hatchery (Victorville, California). After being transferred to the University of California at Riverside, they were maintained in a living stream (Frigid Units, OH, USA) receiving filtered dechlorinated tap water at a flow rate of 5 L/min. The water temperature in the living stream was 14 ± 1°C. The fish were fed Purina rainbow trout chow (St. Louis, MO, USA) at approximately 2% of their body weight every day. Light cycles were at 14:10 h (light:dark). Fish were acclimated to these conditions for at least two weeks prior to exposure.

Water sample collection

The Prado Wetland (33°54" N; 117°40" W) is located in Riverside County, California, USA and consists of 50 shallow ponds that have been utilized to remove nitrogen from water originating from the Santa Ana River, which consists of effluent from tertiary treated wastewater from Riverside County during baseflow conditions (Bachand and Horne 2000). The average flow into the Prado wetland is 80 cubic feet per second. The average depth is 2.5-3 feet and the retention time is 6 days. Water samples were collected at the entrance and exit of the wetland. Water samples from the two sites were collected in 80-gallon Polyvinyl Chloride (PVC) containers in July 2003.

The Green Acres Treatment Plant at the Orange County Water District, Fountain Valley, California, USA treats about 7.5 million gallons of secondary treated wastewater per day from the Orange County Sanitation District (OCSD) using direct filtration followed by disinfection with chlorine. The resultant disinfected water is used for non-portable industrial and irrigation uses in Orange County. The water sample was collected in April 2003 in an 80-gallon container. Since the water sample contained high levels of chlorine (approximately 4 mg/L) and ammonium (approximately 3 mg/L) which were lethal to rainbow trout, sodium thiosulfate was added to dechlorinate the water sample and the water sample was aerated continuously for several days prior to exposure to eliminate chlorine.

In vivo assays

Mixed sexed juvenile rainbow trout were exposed to 9 L of diluted (33%) wastewater samples (3 liters of waste water/6 liters of filtered tap water) in 20 L tanks with aeration in a static renewal system for 14 days. Control fish were exposed to filtered dechlorinated tap water only. Water temperature was maintained at 14 ± 1°C. Light cycle was14:10 h (light : dark). Each treatment (control and exposed) had three replicates with 2-3 fish for each replicate. The water was renewed every other day. Fish were fed rainbow trout chow at 1% of their body weight during the exposure.

In vitro assay (*Yeast estrogen screening*)

One liter of the wastewater samples (from all sampling locations) was filtered over 0.45 and 1.2 um Whatman filters (Clifton, NJ, USA). The water was passed over an Empore SDB-XC extraction disk (Mt. Pleasant, SC, USA). After extraction, the Empore filters were eluted with 30 ml of methanol, with the eluate evaporated to dryness and resuspended in 100 ul of ethanol. The sample was stored at 4°C until use for YES.

The YES assay was performed according to methods previously published (Desbrow *et al.* 1998; Huggett *et al.* 2003). Briefly, 100 ul of the extracted samples or standard concentrations of E2 (10^{-4} – 10^{-14} ng/L in ethanol) were added to 700 ul of a yeast cell suspension (with OD_{600nm} = 0.057) in microcentrifuge tubes. The tubes were incubated with caps open at 30°C for 5 days. The dried sample was resuspended in 100 ul of buffer (60 mM $Na_2HPO_4.7H_2O$, 40 mM $NaH_2PO_4.H_2O$, 10 mM KCl, 1mM $MgSO_4.H_2O$, and 50 mM 2-Mercaptoethanol, pH 7.0) and 400 ul of 10 mg/ml chromogenic substrate (O-nitrophenyl β-D-galactopyranoside, Sigma, St. Louis, MO). Incubation was carried out at 37°C for 1 hour for color development. Absorbance was measured at 405 nm using a microtiter plate reader (model: *Vmax*, Molecular devices, Sunnyvale, California, USA).

The method detection limit of the YES assay was 1 ng/L. Estrogenic activity of the water sample by YES assay was expressed as E2 equivalent concentrations (EEQs).

E2 exposure

Juvenile rainbow trout were exposed to nominal concentrations of E2 of 0 (control, 1ml ethanol only), 0.5 ng/L, 1 ng/L, 10 ng/L, and 100 ng/L in 10 liter of filtered dechlorinated tap water using the same exposure as in the in vivo assays. Measured concentrations were determined as previously described (Belfroid *et al.* 1999; Huggett *et al.* 2003) with minor modifications. One liter of water sample was taken from the exposure tank and filtered with combined 1.2 um Whatman and 0.45 um Millipore filters (Fisher Scientific, Los Angeles, CA). E2 was extracted with the Empore SDB-XC extraction disk previously conditioned with acetone and methanol at a flow rate of 5 ml/min and the disk was eluted with 30 ml of methanol. The extract was evaporated under nitrogen stream until dryness and reconstituted in 0.5 ml of hexane:acetone (65:35, v:v). The resultant mixture was derivatized with 50 ul of Bis-Trimethylsilyl-Trifluoroacetamide (BTSFA, Sigma, St. Louis, MO) at 60°C for 60 min and then evaporated until dryness under stream of nitrogen and reconstituted with 200 ul of hexane. Detection of E2 was performed using an Agilent Technologies 6890N Gas Chromatography System equipped with 5973 Mass Selective Detector (MSD). The capillary column was HP-5MS 30m x 0.25mm, with 0.25 μm film thickness. The GC conditions were: detector - 290°C; ion source, EI mode; injector - 250°C. Column temperature program started at 80°C, isothermal for 2 min; ramped to 200°C with 20°C/min, followed by 2°C/min to 260°C, and held for 10 min. The mass selective detector was used in Selected Ion Monitoring (SIM) mode. The E2 retention time was 23.771 min and the precursor ion was 416 m/z and product ion was 285 m/z.

Analyte recovery was quantified using water samples spiked with 17β-estradiol. The recovery was 60% with a relative standard deviation of 15%. The method detection limit (MDL) was 1 ng/L. The measured concentration of E2 in the solution for the E2 exposure was between 79.9% and 122% of the nominal concentration except for 100 ng/L, for which the measured concentration was 28% of nominal. Analysis of environmental samples for E2 was not carried out due to matrix interference with the analysis (Todorov *et al*. 2002).

Plasma VTG production in juvenile rainbow trout exposed to E2 showed a concentration-related increase after the 0.50 ng/L nominal concentration (R^2 = 0.99, P < 0.0001. Fig.1). The lowest –observed-effect concentration (LOEC) for VTG induction was 1.21 ng/L.

Determination of VTG levels

After termination of the exposure, fish were euthanized in MS-222 (50 mg/L). Blood samples from rainbow trout were obtained by an incision at the caudal peduncle and collecting the blood exiting the incision. Blood was centrifuged at 3000 rpm for 10 minutes at room temperature. After centrifugation, PMSF (Phenylmethyl sulphonyl fluoride; Stock solution 0.1M) was added to the plasma samples at a final concentration of 1mM. The plasma samples were stored at –80^{o}C until analysis.

Vitellogenin concentrations in the plasma were determined using rainbow trout VTG enzyme-linked immunosorbent assay (ELISA) kit supplied by Biosense Laboratories (Bergen, Norway). All assay procedures were followed according to the manufacturer. Briefly, 96-well polystyrene microtiter plates were coated with the capture antibody using 100 ul sodium carbonate coating buffer (50 mM, pH 9.6) per well and incubated at 37^{o}C for 2 hours. After incubation, the plates were washed 3 times with 200 ul per well with phosphate buffered saline (PBS) (20 mM sodium phosphate, 150 mM sodium chloride, pH 7.3) with 0.05% Tween 20. One hundred and fifty ul of blocking buffer (1% of bovine serum albumin in PBS) was added to individual wells of the plates for 1 hour. Next, 100 ul of blocking buffer containing the diluted standard or plasma samples was added to the wells and allowed to incubate at room temperature for 1 hour. After the wells were washed 3 times with 200 ul of washing buffer, 100 ul of the diluted detecting antibody was added to all wells and incubated at 4^{o}C overnight. Secondary antibody in 100 ul blocking buffer was added to each well and the plates were incubated on an orbit shaker (400 rpm) at room temperature for 1hour. After washing, 100 ul of the color development solution (Ellman) was added to each well and the plates were incubated at darkness for 1 hour. The reaction was stopped by adding 50 ul of 2N H_2SO_4 to all wells. The absorbance was read at 405 nm in a microtiter plate reader. Vitellogenin levels in the plasma samples were calculated based on the standard curve obtained from the relationship between the concentration of the standard rainbow trout vitellogenin and the absorbance (for all cases, standard curves have a $R^2 \geq 0.99$).

Total protein levels in the plasma sample were determined using the method of Bradford (Bradford 1976) using bovine serum albumin as standards (0.25 – 2 mg/ml). Plasma samples were diluted in phosphate-buffered saline (pH 7.4) and transferred to 96-well microtiter plates in triplicates. The absorbance was read at 595 nm.

Vitellogenin levels in the plasma samples were expressed as ng vitellogenin per mg of total protein. Estrogenicity of the wastewater samples was expressed as E2 equivalent concentrations as described for YES above.

Statistical Analysis

All statistical analyses were performed using the Statistical Analysis System package (SAS, version 8.0, Cary, NC) unless otherwise stated. Before analysis, data were evaluated for normality with the Shapiro-Wilks test, and for equality of variance using Levene's test. Since assumptions of normality and equal variance (increased standard deviation of the data because of the use of mixed sex juvenile rainbow trout) were violated, data were not transformed and a nonparametric test (Kruskal-Wallis test or Mann-Whitney *U*-test) was used to test the difference in vitellogenin levels between control and treatment groups. The significance level was set at $p \leq 0.05$. For E2 exposure, dose response curves were generated by SigmaPlot software (SPSS Inc, Chicago, Illinois, USA).

RESULTS

Green Acres Plant Assessment

Juvenile rainbow trout exposed to wastewater from the Green Acre Plant for 14 days had a higher level of plasma VTG than control fish. The increase in plasma VTG levels in the exposed fish relative to the control fish was approximately 49-fold. Based on the concentration-response curve ($R^2 = 0.999$.), the VTG E2 equivalent concentration of this tertiary-treated water was 16.92 ± 16.48 ng/L . YES assays indicated that the tertiary-treated wastewater had a YES E2 equivalent concentration of <1 ng/L.

Prado wetland assessment

Juvenile rainbow trout exposed to 33% of the water entering and exiting Prado wetland for 14 days had elevated levels of plasma vitellogenin compared to control fish ($P < 0.05$). The percentage increase in vitellogenin levels in the fish exposed to Prado water relative to the control fish was approximately 21-fold ($P < 0.05$) for entering water and 11-fold ($P < 0.05$) for exiting water. There were no significant differences in plasma vitellogenin levels in trout exposed to Prado influent or effluent water ($P > 0.05$.). VTG E2 equivalent concentrations of the entering and exiting water sample of Prado wetland were 29.80 ± 28.11 ng/L and 24.34 ± 23.17 ng/L. No significant differences in VTG EEQ were observed between water entering and exiting Prado wetland (Fig. 3). YES assays showed that the water samples from the entering and exiting sites had YES E2 equivalent concentrations of 2.57 and <1 ng/L respectively.

Significance:

Wastewaters from the Green Acres Plant had environmental estrogens which induced vitellogenin in juvenile rainbow trout. Entering and exiting water from the Prado wetland also had an estrogenic activity in trout. *In vitro* activity of tertiary-treated water was 10 times less than *in vivo* activity. Wastewater dominated surface water of the Santa Ana River possessed 10-fold greater *in vivo* activity than YES estrogenicity. These data indicate that wetland treatment within the Prado site may not totally alleviate *in vivo* estrogenic activity and ER-based *in vitro* ligand-based assays may underestimate estrogenic activity.

Evaluating the effectiveness of vegetated buffers to remove nutrients, pathogens, and sediment transported in runoff from grazed, irrigated pastures

Basic Information

Title:	Evaluating the effectiveness of vegetated buffers to remove nutrients, pathogens, and sediment transported in runoff from grazed, irrigated pastures
Project Number:	2003CA40B
Start Date:	3/1/2002
End Date:	2/28/2003
Funding Source:	104B
Congressional District:	44
Research Category:	None
Focus Category:	Water Quality, Management and Planning, Surface Water
Descriptors:	None
Principal Investigators:	Kenneth W. Tate, Brad Hall

Publication

1. Publications from 2002 Project Year Bedard-Haughn, A., K.W. Tate, C. van Kessel. 2004. Using 15N to Quantify Vegetative Buffer Effectiveness for Sequestering N in Runoff. J. Environmental Quality. In Press. Publications from 2003 Project Year Bedard-Haughn, A., K.W. Tate, C. van Kessel. Quantifying the Impact of Regular Cutting on Vegetative Buffer Efficacy for 15N Sequestration. J. Environmental Quality. In Review. Bedard-Haughn, A., K.W. Tate, C. van Kessel. Impact of Buffer Management on 15N Attenuation from Surface and Subsurface water. J. Environmental Quality. In Review.
2. Publications from 2003 Project Year Bedard-Haughn, A. 2004. Using 15N to quantify the effectiveness of vegetative buffers for sequestering N. Ph.D. Dissertation, Soil Science Graduate Group, University of California, Davis, CA 95616.

INTRODUCTION: Provide a brief overall introduction to your annual report.

Irrigated pastures are an essential source of low cost, green forage for livestock during summer months when the surrounding rangelands are dry and dormant. California's irrigated pastures are found throughout the Central Valley and Sierra Nevada's, within watersheds providing much of California's surface drinking water supplies. Significant amounts of surface water runoff can be generated from these pastures during irrigation, potentially transporting pathogens, nutrients, and sediment to nearby waterbodies. Our preliminary work indicates that nitrate, phosphorus, fecal coliforms, *E. coli*, *Cryptosporidium parvum* and sediment are transported from these pastures.

Vegetative buffers are often proposed to attenuate pollutants in runoff from grazed pastures. There is general evidence that buffers ranging anywhere from 3 to 200 m wide can protect aquatic resources from adjacent agricultural land use. Buffer recommendations are typically one-size-fits-all, and do not account for pasture characteristics or pollutant loading rates. A more applicable approach would be to base buffer size and management recommendations upon an understanding of the relationships between pollutant load in the pasture, buffer trapping efficiency, and buffer capacity over time for the suite of pollutants common to pastures (N, P, pathogens, and sediment). An understanding of the processes determining buffer efficiency and capacity will lead to more informed buffer establishment and management.

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

1.0. Research Problem

We examined the potential for vegetative buffers positioned at the bottom of flood irrigated, foothill pastures to attenuate nutrients, fecal borne bacteria, dissolve organic carbon and suspended solids contained pasture runoff during irrigation – runoff events.

2.0. Project Objectives

A. Quantify the effectiveness of buffers to attenuate N (NO_3, NH_4, total N), phosphorus (PO_4, total P), a common fecal borne indicator bacteria in livestock (*E. coli*), dissolved organic carbon, and suspended solids in surface water runoff from grazed, flood-irrigated pastures.
B. Employ the N isotope method to quantify N partitioning within pasture, buffer, and runoff.
C. Employ the N isotope method to determine whether buffer capacity for N decreases over time as buffer vegetation matures and species composition changes in the absence of grazing.
D. Extend the results of this research to the livestock industry, UCCE livestock and natural resource advisors, natural resources agency staff and water resource regulators.

3.0. Methodology

Research to achieve Objective A and B were conducted on existing research facilities at the University of California Sierra Foothill Research and Extension Center (SFREC) near Browns Valley in Yuba County. We developed a replicated infrastructure of 9 adjacent flood-irrigated plots allowing for the immediate implementation of this project in 2001. Buffer treatments (see below) were established on these plots in May 2000 and have been maintained continuously since that time. Research to achieve Objective C was conducted on plots established during this grant.

3.1. Objective A and B

During the summers of 2000 and 2001 we established 9 adjacent plots within an existing flood-irrigated pasture. A completely random study design was employed to allocate 3 buffer treatments in 3 replicates to 9 plots. Buffer treatments consists of a 3:1 pasture to buffer area ratio, a 6:1 pasture to buffer area ratio, and a no buffer control. Each plot has a 240 m^2 (5 m wide by 48 m long) pasture area. The 3:1 pasture to buffer area treatment has a buffer area of 80 m^2, and the 6:1 pasture to buffer area treatment has a buffer area of 40 m^2. Buffer length

for the 3:1 and 6:1 buffer treatment is 16 and 8 m, respectfully. Plots were set out perpendicular to the slope and thus irrigation flow. Irrigation water is applied to each plot via adjustable flow rate irrigation pipe. Plots are irrigated for 4 hours every 11 days from April 15 to October 15. No irrigation occurs during the November to March wet season. Irrigation application amount was calibrated to ~ 6.0 and 4.0 L sec^{-1} per plot for 4 hours, in 2002 and 2003, respectively. These rates represent typical application rates for the region. Earthen berms separate adjacent plots to prevent water crossing from one plot to another. PVC collection troughs installed across the bottom of each plot collect all surface water runoff from each plot, allowing for the measurement of surface water runoff and collection of water samples for analysis.

Grazing on pasture areas was by mature beef cattle. Buffer areas and the collection troughs of all plots are fenced to exclude cattle. During 2002 cattle grazing was uniform across all 9 plots, with a grazing event occurring every 30 days, or approximately 1-2 days before every other irrigation event of the season. During 2003 cattle grazing was managed to establish a rest from grazing (days) treatment across the 9 plots such that over 8 trials each plot had at 2, 15, and 30 days rest from grazing prior to irrigation with a minimum of 2 replicates of rest treatment per plot. Each rest duration by buffer treatment combination was present in each trial.

3.1.1. 2003 Trials for Objective A

Eight study trials were conducted in 2003. Trials occurred bimonthly starting June 1. Cumulative cattle fecal load (kg/ha) was quantified in each plot's pasture area the day prior to each study trial via the comparative fecal load method. Buffers were excluded from grazing, fertilization, and all other forms of management. For each plot, surface water runoff (L/s) was measured via stop-watch and graduated cylinder, and water samples were collected at 1 (leading edge of runoff), 15, 30, 60, 90, and 120 minutes following commencement of runoff from each plot during each trial. Plot runoff water samples were analyzed for suspended solids (organic and inorganic), turbidity, pH, electrical conductivity, NO_3, NH_4, total N, PO_4, total P, dissolved organic carbon (DOC), and *E. coli* concentration. Analysis for *E. coli* was conducted within 24 hours of collection at the SFREC laboratory using the standard direct membrane filtration method. The remaining analyses were conducted at UCD following standard methods. Flux (load) of each pollutant per irrigation event was calculated as sum of the mean concentration by flow for each sample period. Linear mixed effects analysis was employed to evaluate buffer treatment effects and effect of rest from grazing and cumulative cattle fecal load on pollutant concentration and flux.

3.1.2. 2002 Use of ^{15}N for Objective B

In June 2002, ^{15}N-labeled KNO_3 was applied at a rate of 5 kg N ha^{-1} and 99.7 atom%^{15}N. The ^{15}N was applied across all 9 plots along the entire width of the experiment. The area to be labeled was 0.5 m wide and located 1 m above the buffer strips. Surface runoff water samples were collected during 12 irrigation trials (11 day schedule) starting 1 day after ^{15}N application. Samples were collected at 1, 15, 30, 60, 90, and 120 minutes following runoff commencement and analyzed for ^{15}N content. Soil water samples within pasture and buffers will be collected after each irrigation event and analyzed for ^{15}N content. Representative plant samples from the pasture and the buffer strips were collected at Days 1, 12, 31, 65, and 86 following application of the tracer and analyzed for ^{15}N content. ^{15}N isotope analyses were performed on all three N pools: NO_3^-, NH_4^+, and total N. To determine how far the ^{15}N-fertilizer moved into the buffer strip, plants were sampled along a down slope transect with a sample spacing of 1 to 2 m and analyzed for ^{15}N isotopic composition. Total aboveground biomass of the vegetation will be determined. Buffers were excluded from grazing, fertilization, and all other forms of management. Linear mixed effects analysis was employed to evaluate buffer treatment effect and effect of time after application and distance down slope from application on ^{15}N concentration and flux.

3.2. Objective C

To determine whether buffer N capacity decreases with increasing maturity of vegetation, a second set of non-grazed, non-fertilized buffer plots were established in the first year of this project (2002). This set contained 10 adjacent plots with the 1:3 buffer treatment (80 m^2, and 16 m buffer length). Among these 10 plots, 5 were allowed to develop to full maturity and 5 will be cut monthly from June through October to allow for maximum

vegetation regrowth. In the second year, ^{15}N will be applied as a tracer to all ten plots, using the methodology outlined above, to determine whether new growth increases the demand for N, thus improving the efficacy of the buffers to trap N in runoff. Soil, soil water, plant materials, and surface runoff were collected as described above and tested for ^{15}N.

4.0. Principle Findings and Significance

4.1. Objective A

Buffers were relatively inefficient at attenuating pollutants under the irrigation application rate and resulting runoff conditions examined in this experiment. Neither buffer treatment resulted in a significant change (increase or decrease) in total flux of *E. coli*, total suspended solids (organic or inorganic), turbidity, total N, nitrate, or total P. This can likely be accounted for by the fact that there was no significant reduction in runoff volume (flux) due to either buffer. Thus, the primary mechanism for buffer efficacy, infiltration, was not sufficient to attenuate these pollutants. The hydrologic transport capacity was too great, and the residency time too short, in the buffer to allow for effective attenuation. There were apparent decreases in total N, nitrate and total P within the buffers indicating some attenuation of these pollutants within buffers and agreeing with results reported for Objective B. However, there were apparent increases in TSS, VTSS, and turbidity, while there were significant increases in DOC ($p=0.048$) at the 3:1 pasture to buffer area treatment. The buffer areas were serving as sources for particulate and dissolved organic matter. Discharge volume (m^3/ha) was positively related to all pollutants, illustrating the dominating influence of hydrologic transport capacity in this system. Duration of rest from grazing (2, 15, and 30 days) prior to irrigation event was significantly related to both the concentration and flux of all pollutants examined. Reduction in pollutant fluxes ranged from 10 to 40% for irrigations events occurring 30 days post grazing compared to irrigation occurring 2 days post grazing.

4.2. Objective B

Regardless of the form of runoff N (NO_3^-, NH_4^+, or DON), more ^{15}N was lost from the non-buffered treatments than from the buffered treatments. The majority of the N attenuation was by vegetative uptake. Over the course of the study, the 8 m buffer decreased NO_3^--^{15}N load by 28% and the 16 m buffer decreased load by 42%. For NH_4^+-^{15}N, the decrease was 34% and 48%, and for DON-^{15}N, the decrease was 21% and 9%. Although the buffers were effective overall, the majority of the buffer impact occurred in the first four weeks after ^{15}N application, with the buffered plots attenuating nearly twice as much ^{15}N as the non-buffered plots. For the remainder of the study, buffer effect was not as marked; there was a steady release of ^{15}N, particularly NO_3^--^{15} and DON-^{15}N, from the buffers into the runoff. This suggests that for buffers to be sustainable for N sequestration there is a need to manage buffer vegetation to maximize N demand and retention.

4.3. Objective C

Although maximum plant ^{15}N uptake and sequestration occurred within the zone of ^{15}N application, over-cutting of the pasture vegetation led to belowground N losses. Some of this ^{15}N was subsequently immobilized within the microbial biomass further down slope, but was still a potential source for leaching or runoff N losses. In the buffers, the cutting effect was not significant in the first few weeks following ^{15}N application, but over the irrigation season, cut buffers sequestered 2.3 times the ^{15}N of uncut buffers corresponding to an increase in above-ground biomass following cutting. Cutting and removing vegetation allowed the standing biomass to take advantage of soil ^{15}N as it was released by microbial mineralization. In contrast, the uncut buffers showed very little change in ^{15}N sequestration or biomass, suggesting senescence and a corresponding decrease in N demand. The doubling of ^{15}N sequestration in the cut buffers confirms that regular cutting and harvest of buffer vegetation increases N demand and uptake and thus, vegetative buffer effectiveness.

The greatest effect of cutting on runoff ^{15}N concentration occurred in the NO_3^- and total dissolved N pools: the uncut buffers had higher ^{15}N concentrations than the cut buffers. This same trend could be observed throughout the duration of the experiment in the NH_4^+ and DON pools. Soil solution ^{15}N concentrations in the A horizon were also significantly higher in the uncut buffer than in the cut buffer; however there was no corresponding significant difference in 0-15 cm soil atom % ^{15}N excess. Overall, cutting significantly improved ^{15}N attenuation from both surface and subsurface water. However, the effect was temporally related, and only

became significant 21-42 d after ^{15}N application. The dominant influence on runoff water quality from irrigated pasture remains irrigation rate: reducing the rate by 75% relative to the typical rate resulted in a 50% decrease in total runoff losses and a 7-fold decrease in ^{15}N concentration.

4.4. Overall Significance

These results clearly illustrate that under typical high rates of irrigation application and resulting runoff it is unreasonable to expect significant attenuation of pollutants in runoff by non-grazed vegetative buffers installed at the base of flood irrigated foothill pastures. The hydrologic transport capacity and flushing ability of these high runoff rates reduce infiltration and residence time of pasture water entering the buffer and serve to mobilize and transport pollutants contained within the buffer. These results clearly illustrate that improvement of runoff from these pastures can be achieved primarily through integration of improved irrigation efficiency to reduce runoff and grazing management designed to off-set the grazing events from irrigation events, with the secondary implementation of managed (cut or moderately grazed) buffers once runoff rates delivered to the buffer are in balance with the buffer's infiltration capacity. Regular removal of vegetation in buffers is critical to maintain the buffer vegetation's nutrient uptake capacity and to reduce the build up of organic matter which can serve as a source for VTSS and DOC. Our future work in this system will examine the efficiency of buffers under irrigation application timing and rate determined by plant water demand and soil infiltration capacity in conjunction with multiple grazing management scenarios.

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

This study has played a major role in the University of California's *Rangeland Watershed Program's* (RWP) extension activities over the past several years. Since 1997 the *Ranch Water Quality Planning Short Course* conducted by the RWP has helped more than 400 ranchers voluntarily complete water quality plans covering more than 1 million acres of private ranches. The results of this research have been integrated into the numerous extension activities we conduct as part of the RWP including the ranch water quality short course. UC SFREC, the project site, has a mandate for extension education and information transfer of all research knowledge generated on the facility. We have been active in conducting field tours at the project site and have participated in the SFREC annual field day which is attended by ~150 individuals each year. In addition, the project site will be the site of a 2 day workshop on vegetative buffers to be held in Fall 2004 for agriculturalists from Chile. Finally, the results of this project have been reported nationally via presentations at professional meetings and conferences.

Extension presentations, field tours, professional presentations include:

2003 and 2004. SFREC Annual Field Day – site was a stop on the annual field tour and presentations were made to the group by project leaders and graduate students.

2003. CA Society for Range Management field tour at SFREC - site was a stop on the field tour and presentations were made to the group by project leaders and graduate students.

2003. Results were reported to CA State Water Resources Control Board – Surface Water Ambient Monitoring Program Continuing Conference.

2003. USDA NRCS Continuing Education Field Day - site was a stop on the field tour and presentations were made to the group by project leaders and graduate students.

2003. Bedard-Haughn, A., Tate, K.W., and van Kessel, C. Attenuation of nitrate-^{15}N by vegetated buffers in an irrigated pasture system. American Geophysical Union Fall Meeting, San Francisco, CA. Oral presentation

2003. Bedard-Haughn, A., Tate, K.W., and van Kessel, C. Vegetative buffer efficiency in an irrigated pasture system. Canadian Society of Soil Science Annual Meeting, Montréal, QC. Oral presentation.
Awarded: C.F. Bentley Student Presentation Award for Excellence in Oral Presentations (1st place)

2004. Bedard-Haughn, A., Tate, K.W., and van Kessel, C. Increasing the demand: The impact of regular cutting on vegetative buffer ^{15}N uptake. Soil Science Society of America and Canadian Society of Soil Science Joint Annual Meeting, Seattle, WA. Oral presentation.

2004. Tate, K.W., Bedard-Haughn, A., and van Kessel, C. Sink or source? Managing vegetative buffers to minimize N in runoff. Soil Science Society of America and Canadian Society of Soil Science Joint Annual Meeting, Seattle, WA. Oral presentation.

2004. Bedard-Haughn, A., Tate, K.W., and van Kessel, C. Using ^{15}N to quantify vegetative buffer efficiency for sequestering N in runoff. Ecological Society of America Annual Meeting, Portland, OR. Oral presentation.

2004. Bedard-Haughn, A., Tate, K.W., and van Kessel, C. Using ^{15}N to quantify vegetative buffer efficiency in an irrigated pasture system. Riparian Ecosystems and Buffers: Multi-scale Structure, Function, and Management. American Water Resource Association, Summer Specialty Conference, Olympic Valley, CA. Oral presentation.

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.

Please fill in the table below where applicable.

	Total Project Funding		Supplemental Awards	Total
	Federal Funding	State Funding		
Undergrad.	5,364.51			5,364.51
Masters				
PhD.	1,956.73			1,956.73
Post-Doc.				
Total	7,321.24			7,321.24

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.

Tate, K.W., E.R. Atwill, C. van Kessel, J. Six, R.A. Dahlgren. 2004-2008. Implementation of Vegetative Buffer, Irrigation, and Grazing Best Management Practices to Reduce Pathogens, Organic Carbon, and Colloids in Runoff from Rangelands and Irrigated Pastures. CALFED Proposition 50 Drinking Water Quality Program. $886,133.

Jastro-Shields Graduate Research Scholarship, University of California, Davis. 2003 ($1,600), 2002 ($2,200).

UC Davis Dissertation Year Fellowship, University of California, Davis (2003-2004)

C.F. Bentley Student Presentation Award for Excellence in Oral Presentations (1st place), Canadian Society of Soil Science Annual Meeting (2003)

Publications from 2002 Project Year

Bedard-Haughn, A., K.W. Tate, C. van Kessel. 2004. Using ^{15}N to Quantify Vegetative Buffer Effectiveness for Sequestering N in Runoff. J. Environmental Quality. In Press.

Publications from 2003 Project Year

Bedard-Haughn, A. 2004. Using ^{15}N to quantify the effectiveness of vegetative buffers for sequestering N. Ph.D. Dissertation, Soil Science Graduate Group, University of California, Davis, CA 95616.

Bedard-Haughn, A., K.W. Tate, C. van Kessel. Quantifying the Impact of Regular Cutting on Vegetative Buffer Efficacy for ^{15}N Sequestration. J. Environmental Quality. In Review.

Bedard-Haughn, A., K.W. Tate, C. van Kessel. Impact of Buffer Management on ^{15}N Attenuation from Surface and Subsurface water. J. Environmental Quality. In Review.

California Water Resources Center WRIP Program

Basic Information

Title:	California Water Resources Center WRIP Program
Project Number:	2003CA44B
Start Date:	3/1/2003
End Date:	2/28/2004
Funding Source:	104B
Congressional District:	44
Research Category:	None
Focus Category:	Education, None, None
Descriptors:	None
Principal Investigators:	John Letey, Martha Kennedy

Publication

The mission of the Water Resources Center is to stimulate and support water and water related research both within and among the various academic departments and research organizations of the University of California. The broad research focus includes the conservation, development, management, distribution, and utilization of water resources with a view to their optimum present and future use for the State of California. Dr. John Letey is head of program administration/management. Four research projects from the University will share in the grant this year. They are: Dr. Ken Tate, UCD, "Evaluating the effectiveness of vegetated buffers to remove nutrients, pathogens, and sediment transported in runoff from grazed, irrigated pastures"; Dr. Daniel Schlenk, UCR, "Use of bioassays to assess the water quality of wastewater treatment plants for the occurrence of estrogens and androgens"; Dr. Jan Gan, UCR, "Pyrethroid insecticides in nursery runoff: Transport and impact on aquatic invertebrates"; and Dr. Paul De Ley, UCR, "Structure and seasonal changes of nematode communities from vernal pools (Santa Rosa Plateau)".

Dynamic Chemical Loads as a Function of Land-Use Changes in a Watershed

Basic Information

Title:	Dynamic Chemical Loads as a Function of Land-Use Changes in a Watershed
Project Number:	2000CA6G
Start Date:	9/1/2000
End Date:	8/31/2002
Funding Source:	104G
Congressional District:	43
Research Category:	Not Applicable
Focus Category:	Surface Water, Solute Transport, Management and Planning
Descriptors:	
Principal Investigators:	Arturo A Keller

Publication

TITLE: Dynamic Chemical Loads as a Function of Land-Use Changes in a Watershed

Principal Investigator: Arturo A. Keller
Bren School of Environmental Science & Management
University of California, Santa Barbara, California 93106
keller@bren.ucsb.edu
(805) 893-7548

Key Words: Fertilizers Nutrients, Pesticides, Toxics, Buffer Strips, Contaminant Transport, Decision Models, Land Use, Landscape Management, Land-Water Interactions, TMDL, Water Quality Modeling, Watershed Management

Focus Categories: NPP, WQL, MOD

The basins model is about 80 per cent ready and the MMS model is approximately 50 per cent ready.

There have been no publications to date

Training: Eight graduate (masters) students and one PhD student have been directly involved in the implementation of watershed models.

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, 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).

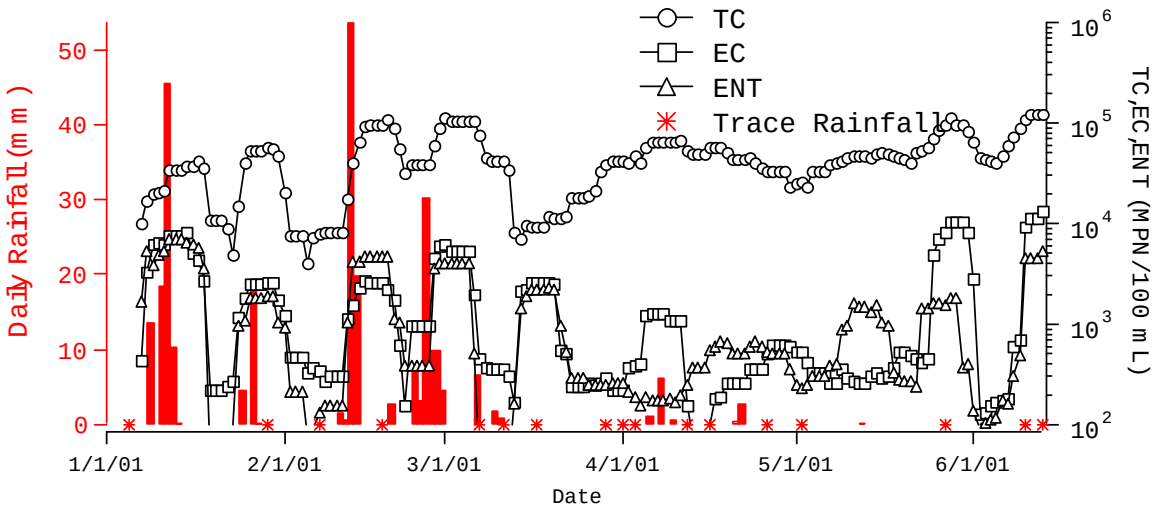

Figure 1. Daily FIB concentrations measured in the Fountain Valley Channel, which drains a coastal urban community in the Santa Ana River Watershed. Water from this stream was collected every day for six months beginning January 1, 2001. Samples were analyzed for Total Coliform (TC), *E. coli* (EC), 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.

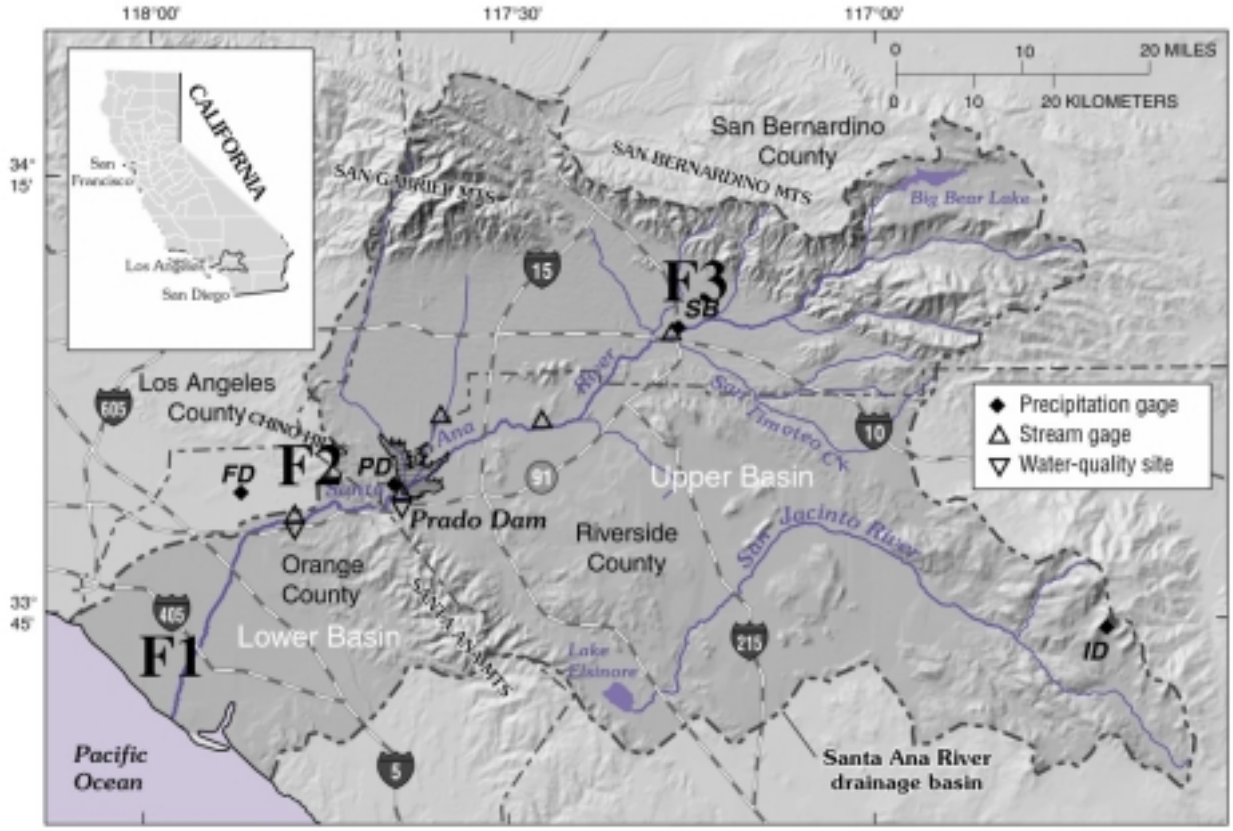

Fig. 2. A map of the SAR watershed. Proposed field sites are indicated by number: F1. Fountain Valley Channel, F2. Imperial Highway, and F3 Warm Creek.

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]
	SONDJFMAMJJASONDJFMAMJJAS
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.

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Information Transfer Program

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 RCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	7	0	0	7	14
Masters	0	0	0	0	0
Ph.D.	2.5	0	0	2.5	0
Post-Doc.	3	0	0	3	6
Total	10	0	0	10	20

Notable Awards and Achievements

Tate, K.W., E.R. Atwill, C. van Kessel, J. Six, R.A. Dahlgren. 2004-2008. Implementation of Vegetative Buffer, Irrigation, and Grazing Best Management Practices to Reduce Pathogens, Organic Carbon, and Colloids in Runoff from Rangelands and Irrigated Pastures. CALFED Proposition 50 Drinking Water Quality Program. $886,133. Jastro-Shields Graduate Research Scholarship, University of California, Davis. 2003 ($1,600), 2002 ($2,200). UC Davis Dissertation Year Fellowship, University of California, Davis (2003-2004) C.F. Bentley Student Presentation Award for Excellence in Oral Presentations (1st place), Canadian Society of Soil Science Annual Meeting (2003)

Publications from Prior Projects