

First Annual Student Research Poster Symposium

**May 16, 1997
1 - 4 p.m.
Holt Hall 111, 113 & 125**

**California State University, Chico
Department of Biological Sciences**

CLASS PROJECTS -- Holt 125

The effect of acid rain on the growth of bean seedlings

Chris Ames, Eric Doyne, Jennifer Moore, and Shelley Roberts
Biology 6B - Sponsored by Ms. Shelley Kirm

Acid deposition is extensively publicized as one of the most serious environmental threats known today, and acid rain being a form of this deposition is of enormous environmental and economic importance. In a normal unpolluted environment, rainwater is sparsely acidic with a pH level of 5.7. Recently documented pH levels of acid deposition ranging between 1.69-3.9 in the Los Angeles basin sparked our inquiry to whether a simulated experiment could imitate acid rain, and what the results would be.

Acid rain is formed when two pollutant gases, sulfur and nitrogen oxides combine with water in the atmosphere and then fall to the earth as precipitation. Sulfur oxides form sulfuric acid in the form of acid rain, thus sulfuric acid will be used in our study.

Acid rain has been determined to cause foliar damage, impair seed germination, erode waxes, and leach nutrients from plant leaves. Chiras, in these statements provided a means to test for ourselves whether this can be replicated with the same results. Our experiment will imitate the effects of a sulfuric acid solution with pH values between 2-3 on the growth rate of bean seedlings.

Through our experiment, we hope to show that the introduction to the normal growth cycle of bean seedlings will prove these statistics correct, that acid rain will impair growth, and also cause damage to the plants. Our control plants will illustrate the normal growth rate, and the treated plants will illustrate the harmful effects of the acidic solution.

The effects of UV radiation on bacterial growth

Dahl Bradford, Carrie Furman and Rod Lacey
Biology 6B - Sponsored by Dr. Ailsie McEntegart

With the reduction in the ozone in the stratosphere, more solar radiation is reaching the earth, and this trend can be expected to continue for some time. Of specific concern are increases in the amounts of UV radiation reaching the biosphere, or surface. UV radiation lies between 260-280 nm, and this specific wavelength has been shown to disrupt the normal replication of DNA. So with global increase in UV levels, concern is increasing about the effect this will have on biological communities.

In this experiment, we have explored the effect that varying levels of UV radiation have on life in the simplest form, a bacterial cell. If the genetic structure of the cell is damaged, this will eventually lead to its inability to carry out normal

functions, like reproduction, and more damage still would lead to the death of the cell. The purpose of this experiment was to observe and measure the effect of UV radiation has on the growth of the bacteria *E. coli*.

***Felis catus catus*, What are you at-us? (Territoriality and behavior of domesticated felines)**

John Calvin

Biology 258 - Sponsored by Dr. Robert Schlising

Domesticated animals play a large role in the lives of humans. They bring us joy, as well as performing tasks such as guarding dwellings and keeping them free of rodent infestations. This study is primarily concerned with the behaviors, and territories, of domesticated cats, or *Felis catus catus*.

Over fourteen cats were observed during a seven-week period, from April 29, to May 8, 1997, in order to determine if their overall behavior patterns changed within that time. The study was performed at Paradise Apartments, Chico, A, for a number of reasons. The apartment complex is large and densely foliated, allowing for a great diversity of non-domesticated organisms. The complex has three separate courtyards, each including a pool, and of course, there is an abundance of felines. The study area was centralized around the northwest courtyard.

Behaviors of the felines were expected to change, over the seven-week period, due to seasonal changes. Several distinct behaviors were observed in the feline population including the marking and sharing of territory, confrontations both physical and non-physical, hunting and stalking of prey, and incursions of territory by transitory felines.

This work gives some insight into the behaviors and of a fairly large population of domesticated felines. This work also reveals information about how individuals in such a population interact with one another.

Electron microscopic analysis of a biodegradative bacterium.

John Calvin and Cari Jung

Biology 295 - Sponsored by Dr. Richard Demaree

Bacteria that degrade natural and man-made environmental pollutants have been studied extensively, and much is known about their degradative abilities. We were primarily concerned with cell wall and cell membrane morphology of bacteria that degrade petroleum hydrocarbons and para-nitrophenol (PNP). These bacteria are believed to have unique morphologies, which may aid their survival in the presence of pollutants.

We examined the ultra structure of several degradative bacteria using scanning and transmission electron microscopes. Bacterial samples were prepared using standard glutaraldehyde/osmium tetroxide fixation techniques.

Our results yielded a high degree of variation in ultrastructure between the different bacterial specimens. The presence of pili was expected, since petroleum degradative abilities are often coded for on transferable plasmids. It was not surprising to find capsules, which are used by many bacteria to protect from desiccation or to block the entry of toxic substances. Differences in the gram- and gram+ cell wall structure were noted. The effects of PNP on the morphology of PNP degrading bacteria were also examined. Filamentous arrangements were seen in PNP degrading bacteria grown without PNP, while those exposed to high levels of PNP (0.2 mM) did not form filaments.

This work gives insight into how bacteria may be capable of surviving in otherwise lethal conditions through the excretion of capsular material or other physiological adaptations. This work also displays the physical effects of a pollutant (PNP) on bacteria, and gives us a visual comparison of gram- vs. gram+ cellular morphology.

The effect of temperature on bacterial levels in Big Chico Creek

Josh Griggs, Jared Jenkins, and Marty Mares

Biology 6B - Sponsored by Dr. Ailsie McEntegart

The effect of temperature on bacterial levels in Big Chico Creek was studied by taking water samples at different temperatures and growing bacterial cultures on nutrient agars. The colonies were then counted to provide a measure of the numbers of bacteria present in the water samples. It was hypothesized that as the water temperatures in Big Chico Creek increased, bacterial levels would also increase.

The Effects of Kinetin treatment on monocotyledons and dicotyledons

Dan Holochwest, Maria Moran and Kevin McMartin

Biology 330 - Sponsored by Dr. James Pushnik

The purpose of this experiment is to determine if foliarly applied Kinetin can promote growth in dicotyledons and monocotyledons. Kinetin is a hormone that promotes cell division, shoot proliferation, and shoot morphogenesis. To investigate the effects of Kinetin on monocotyledons and dicotyledons, a study was conducted on *Zea mays*(corn) and *Phaseous vulgaris*(bean). External application of Kinetin may be a possible benefit to agriculture by promoting crop growth and yield. Seeds were germinated and then grown in pots with soil. Two control groups and ten treatment groups of plants culled to similar size were used. Five different concentrations of Kinetin were used: 0.01, 0.1, 1.0, 10.0, and 100.0 µg/ml. The different concentrations of Kinetin were foliarly applied three times a week. The plant heights were measured every day for eleven days. The results that we expected were that both the corn and beans would show an increase in growth from the Kinetin treatments and the highest concentration of

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Kinetin would show the most growth. The actual results indicate that the corn and bean plants have a similar response in that their growth was inhibited by Kinetin.

Correlation between number of seed pods and leaf diameter of small and large plants, and death rate (longevity) of *Montia perfoliata* (Miner's Lettuce)

Michelle McFarland

Biology 258 - Sponsored by Dr. Robert Schlising

This study on *Montia perfoliata*, Miner's Lettuce, was done from March 14, 1997 to May 8, 1997, in the 5-mile recreation area of Upper Bidwell Park. The purpose of the study was to determine whether or not there is a correlation between leaf diameter and the number of seed pods. The null hypothesis was that there is no difference in number of seed pods between "small" and "large" plants. Sixty plants were measured from three spots, the average leaf diameter being 4.19 cm. Those with a diameter less than the mean were designated "small", and those with a diameter greater than the mean were designated "large". A t-test was done to compare the mean ($\bar{x} \pm \text{s.d.}$) number of seed pods in "small" (10.29 \pm 2.11) and "large" (13.83 \pm 2.73) plants. The result was a t-value of 5.6 compared to the Table t-value of 2.66. From this information the null hypothesis was rejected, showing that there is a very significant difference ($\alpha = 0.01$) in number of seed pods based on leaf diameter. The study also included an analysis of death rate of the plants in one spot. Over the time period of the study (26 days), the plants died at a mean rate of 1.92 per day.

An Analysis of Green Swallowtail chrysalis distribution on cement pillars in Bidwell Park, and the emergence pattern of the butterfly, the caterpillar, and the "new" chrysalises

Andrea White

Biology 258 - Sponsored by Dr. Robert Schlising

The Green Swallowtail butterfly (*Battus philenor*) is a common site in Chico and in the Bidwell park. The chrysalises of the Green Swallowtail, or more commonly known as the Pipevine Swallowtail, can be found attached to the cement pillars that support Hwy. 99 and run through a portion of Bidwell park. These pillars are arranged in parallel rows, with sides facing north, south, east and west. There is a clearing between the two rows through which light filters. However, each face of the pillars, and each row, receive varying amounts of light and ivy growth. The number of Green Swallowtail chrysalises found on each face of the pillars and in each row also varies. It is this distribution pattern that is analyzed, along with the pattern of emergence of the butterflies, their caterpillars, and the resulting "new" chrysalises.

The emergence patterns were tracked through time showing the time frame in which the butterflies reproduce and new chrysalises are formed. The time observed gives evidence for a second generation. The distribution patterns seen show more chrysalises on the west face of the east row of pillars, and on the east face of the west row of pillars. This indicates a possible "preference" for either light or ivy as they occur simultaneously. This pattern was observed for both last years chrysalises, and this years first generation of Green Swallowtail caterpillars.

The Uptake and localization of lead in *Pinus ponderosa* seedlings.

Natalie Wight and Jeffrey Gauct

Biology 330 - Sponsored by Dr. James Pushnik

The toxicity of lead and its effects on physiological processes of higher plants is an increasing focus of study in developmental plant biology. Lead uptake by such plants may someday assist in the removal of lead from the environment via bioremediation. As such, it is important to understand the localization of the lead into young plants. In this study, young, soil-grown, *Pinus ponderosa* seedlings are treated with various concentrations of lead, are divided into root, stem and needle parts, nitrate/perchlorate acid digested and analyzed using atomic absorption spectroscopy. Due to the nature of the experiment, a higher level of lead would be expected to accumulate within the roots of the plants, which were in immediate contact with the contaminated soils. It will be interesting to learn, however, whether lead has been deposited in the above ground stem and needles of the seedlings.

**INDEPENDENT STUDY PROJECTS
BIOLOGY 199 - Holt 113**

Adenoviral-Mediated Gene Therapy for the Treatment of Vascular Restenosis

Jeanette A. Ardans

Sponsored by Dr. R. Thomas, NIH preceptors: M. Jones, MD, A. Nunez, MD, H. Chikada, MD, I. Yamada, MD

Vascular restenosis, characterized by the excessive proliferation of smooth muscle cells within the vessel, results from the injury induced by such disease treatments as balloon angioplasty. This protocol investigated the use of an adenoviral-mediated gene transfer system to inhibit restenosis in the coronary arteries.

A team of cardiovascular surgeons at the NIH performed the in vivo portion of the protocol by grafting bilateral saphenous vein-carotid artery grafts in domestic swine. Two weeks post-grafting, the health of the grafts were established followed by adenovirus transfection, stent implantation and follow-up measurements.

The in vitro protocol sought to investigate the effects of adenovirus on vascular smooth muscle cells when transfected in tissue culture. The variables under investigation included the time of adenovirus to smooth muscle cell contact (1-hour, 3-day), vascular smooth muscle cell type (arterial, venous), adenovirus type (Ad.RSV.CD, Ad.RSV.Bgal), and five media (.01, .1, 1.0 mMol 5-fluorocytosine + M199+10% FBS, 1.0 mMol 5-fluorouracil + M199+10% FBS, or "mock", M199+10% FBS). All cell counts were performed in triplicate and cultures were counted on Day 0,2,4,6 and 8.

At present time publication is pending and no data have been released to the general public. For this reason, the emphasis of this poster will be on the experimental protocol, my role as a student intern in cardiovascular surgery and the methods employed for the in vitro experiment.

Golden Clover--an initial investigation

Catherine Bishop

Sponsored by Dr. Robert Schlising

Trifolium jokerstii is a gold colored annual clover whose natural history has not been studied. Once considered a variant of *Trifolium barbigerrum* var. *andrewsii*, it has now been proposed for separate species status. The study site is located in an area owned by the state of California on Table Mountain near Oroville. The area contains most known populations. The clover grows exclusively in vernal swales on a substrate of 30-40 million year old volcanic basalt. To gain insight into reasons for its extremely restricted range, this initial study focused on reproductive potential and microsite characteristics. On 5 April 1997 a sample grid 8m X 8m was lined out covering a single population. Random numbers were used to choose points on the gridline within each 1-meter interval (72 total points). The number of flower heads on the plant nearest each point were counted (mean heads per plant: 2.6 +/- 1.4). Two flower heads from each sample point were picked and examined to determine average total flowers per head (9.4 +/- 2.7). Average number of pollinated flowers per head was also determined (7.7 +/- 3.1, 81%). On 12 April at 20 random points along transect lines, the closest mature flower head was enclosed in lens tissue. Of the 10 heads that were recovered two weeks later, the average number of mature seeds per head was determined (3.4 +/- 3.2, 32%). At the same sample points the number of plants in a 20cm X 20cm (.04 sq. m) area was counted. Plant density was calculated to be 324 plants per sq. m. Analysis of soil, and a list of

associated plant species was made. The state management plan for this property is in the initial stages. These data on a rare plant will be submitted for consideration in any activities planned for the site.

p-Nitrophenol degradation in cell free extracts from *Arthrobacter aureescens* TW17

Dan Clark

Sponsored by Dr. Larry Hanne, Department of Biology and Dr. Larry Kirk, Department of Chemistry

Category winner

p-Nitrophenol (PNP) is a toxic by-product resulting from microbial hydrolysis of the organophosphate pesticide parathion. Previously several bacterial strains capable of degrading PNP (including *Arthrobacter aureescens* TW17) were isolated from agricultural soil in northern California. While much is known about the genetics of PNP degradation in TW17, little is known about the enzyme(s) involved in the process. The three main objectives of this study were: 1) to establish conditions for maximal expression of the enzyme involved in the first step of the PNP degradative pathway, 2) to demonstrate cell free activity of this enzyme, 3) to determine the effect of various coenzymes on PNP degradation by this enzyme in cell free extracts.

Maximal enzyme expression was determined by inducing TW17 1-4 times with PNP followed by a final addition of PNP that was then tracked spectrophotometrically. Enzyme activity was demonstrated by incubating cell free extracts with PNP followed by qualitative colorimetric nitrite assays (nitrite is released during PNP degradation). The effects of NADH, NADPH, and FAD on PNP degradation were determined spectrophotometrically by observing the decrease in absorbance for PNP over time in filtered cell free extracts containing added coenzyme.

The results of this study showed that maximal enzyme expression occurred after 3 or 4 inductions with PNP. Cell-free activity was demonstrated by the detection of nitrite in filtered extracts that had been incubated with PNP. Furthermore, addition of FAD to extracts showed the highest PNP degradation rates over the other coenzymes tested.

Future Forest Growth: effect of increased CO₂ and varying water regimes on the morphology of *Pinus ponderosa*.

Michael Lennox

Sponsored by Dr. James Pushnik

Little information is known about the effect of global climate change on organisms. A three year climate change experiment has recently ended involving seedlings of *Pinus ponderosa* grown in the expected CO₂ concentrations of 50

and 100 year atmospheres (525 $\mu\text{L/L}$ and 700 $\mu\text{L/L}$ respectively). The hypothesis established expected the seedlings to acclimate to the environmental change by altering growth rates and patterns of carbon allocation. This study focused on morphological growth performance by measuring (1) the effect of increased atmospheric CO_2 , (2) response to alterations in available soil moisture, and (3) the interaction between both CO_2 and water. Bimonthly growth measurements were taken over a year with respect to terminal elongation and caliper expansion for the seedlings grown in controlled chambers at CSU, Chico ($n=84$). Compared with controls (350 $\mu\text{L/L}$), growth rates were found to decrease with increasing CO_2 . Depending on percentage soil moisture, biomass measurements show a moderate decrease in carbon accumulation and crown surface area, while the total surface area of fine roots ($< 2\text{mm}$) increased. Parallel studies on seedlings grown in open-topped chambers at Lawrence Livermore National Laboratory found both contradictory and supportive data ($n=124$). Preliminary conclusions find altered allocation and growth patterns to exist in environments of elevated CO_2 .

Riparian Vegetation Survey

Mike Lennox and Chris Christoffersen

Sponsored by Dr. Paul Maslin

A survey of the vegetation along Big Chico Creek was conducted during the fall, 1996 to investigate the affects of the diversion of water from Big Chico Creek at five mile on riparian flora. The weir at Five Mile Recreation Area diverts water from Big Chico Creek into Lindo Channel and Sycamore Creek during times of high water. The decrease in water may cause modifications in the flora due to flood repression. Fifteen sites were sampled, approximately one mile apart, from the confluence at the Sacramento River to Salmon Hole in "Iron Canyon" of Bidwell Park. At each site, 20 meter long by two meter wide transects was sampled on both sides of the creek bank. Woody species were quantitatively recorded in terms of diameter-at-breast height (DBH) while non-woody species were qualitatively observed. The Null Hypothesis is that there is no difference in the number of exotic plant species above and below the diversion weir at Five Mile Recreation Area. Results suggest that a significant difference exists between the numbers of exotic plant species above and below the weir.

Transposon mutagenesis of p-nitrophenol degradative gene(s) previously cloned from *Arthrobacter aureus* strain TW17

Jon C. Marlowe, Jeff McGie, Simon Keath, Rich Miller

Sponsored by Dr. Larry Hanne

p-Nitrophenol (PNP) is a by-product of pesticide degradation and is listed by the EPA as a high priority pollutant. Previously, several strains of *Arthrobacter* that could degrade PNP were isolated from soil samples taken from local walnut orchards. PNP degradative gene(s) from these strains were found to be encoded by a 60kb plasmid and localized to a 5kb and 7kb fragment on that plasmid. These fragments have been cloned into an *E. coli* plasmid pBlueSK+ and transformed into *E. coli*. In order to more precisely map the location of PNP degrading gene(s), a library of Tn5 (which codes for kanamycin resistance) mutants with the 5kb and 7kb fragments was generated. Suicidal lambda::Tn5 phage was used to deliver the Tn5 into *E. coli* JM83 containing either the 5kb or 7kb fragment. JM83 in which Tn5 had transposed to the plasmid or chromosome were selected on kanamycin media. In order to isolate plasmids containing the Tn5, plasmid DNA from the entire population of these JM83 mutants was purified and electroporated into *E. coli*. The position of the transposons within the plasmids was then characterized by restriction mapping. We will report results of the locations of Tn5 in the 5kb and 7kb fragments.

GRADUATE STUDENT PROJECTS – Holt 111

The biochemical response of Sucrose Phosphate Synthase in *Pinus ponderosa* exposed to elevated atmospheric CO_2 .

Scott M. Bauer

Sponsored by Dr. James Pushnik

The greenhouse effect on plants is of global concern. Carbon dioxide (CO_2), a major contributor to the greenhouse effect is expected to double in the next century. The impact of increasing CO_2 on long-lived species unable to migrate in response to these changing climatic conditions is unknown. To assess the potential of this impact on ecological systems, seedlings of *Pinus ponderosa*, a canopy dominant species, were grown in environmentally controlled chambers under ambient (350 $\mu\text{L L}^{-1}$) and increased carbon dioxide conditions (525 $\mu\text{L L}^{-1}$ and 700 $\mu\text{L L}^{-1}$) for 9 months. The pine seedlings exhibited distinct changes in carbon allocation patterns reflected in both the growth and physiology. The mechanism for the observed growth changes lies in the biochemistry of photosynthetic carbohydrate metabolism.

The principle objective of this study was to evaluate a major control point in plant carbon metabolism, sucrose-phosphate synthase (SPS). SPS is metabolic regulatory point in sucrose synthesis which is responsive to feedback mechanisms which couple carbon assimilation with growth and physiological demands. Increased activity would reduce the negative feedback on the enzyme thereby allowing higher rates of carbon assimilation and increased partitioning of carbon to plant growth points. These studies revealed that in *Pinus ponderosa* total needle soluble protein concentrations increased at the highest CO₂ exposure level (700 μ L L⁻¹ CO₂). SPS specific activity (μ mol sucrose formed /min/mg protein /cm² needle surface) showed significant ($P < 0.05$) at both levels of elevated CO₂. Kinetic analysis of the enzyme activities showed significant increases in the apparent V_{max} , while no change was observed in the K_m . Significant changes in the SPS activation states suggest that the trees are modulating SPS activity by adjusting both activation states and SPS translation.

These results imply that selective breeding or biotechnological enhancement of SPS activity in a long-lived species such as *Pinus ponderosa* may allow terrestrial carbon banking efforts to succeed and assist in the mitigation of atmospheric carbon build-up.

Seasonal Variation in Freeze Tolerance and Cryoprotectant Synthesis of the Pacific Tree Frog *Hyla regilla*

Category
winner

Scott Adam Croes

Sponsored by Dr. Robert Thomas

Since 1982, freeze tolerance has been discovered in five species of frogs. The current study examined the effect of freezing on *Hyla regilla*, which had not previously been investigated. Frogs were collected in the Sierra Nevada Mountains of Northern California at an elevation of 6,000 feet and tested in the spring and fall. Freezing response was measured in terms of survival, metabolite levels of plasma, liver, and muscle, supercooling/freezing point, and plasma osmolality. Specimens frozen at -2C for 6 and 12 hours had a survival rate of 100% and 80% respectively, in both the spring and fall. Freezing caused a significant 5 fold increase in plasma glucose in the spring and a 14 fold increase in the fall. Levels of liver glucose and glycerol also increased with greater amounts of glucose being produced in the fall. As expected, this rise in glucose was accompanied by a significant decline in liver glycogen. Seasonal differences in muscle glycogen levels in response to freezing was not shown. There was no seasonal differences in supercooling/freezing point or plasma osmolality.

This study represents the first report of freeze tolerance in *Hyla regilla*. The significant rise in plasma glucose, liver glucose, and liver glycerol in response to freezing suggests these compounds are probably being used as cryoprotectants with glucose being the primary component.

An investigation of Rock and Mud Creek, Butte County, California

Jody M. Galloway

Sponsored by Dr. Paul Maslin

The primary purpose of this project was to investigate stream characteristics of Rock and Mud Creeks, identify fish species, determine the geographic distribution of the fish species present, determine their relative abundance at various sampling sites, and identify aquatic invertebrates that inhabit Rock Creek and an adjacent spring. Little scientific information regarding stream characteristics and biotic communities has been gathered from these creeks. While one can infer many biological assumptions about these creeks based on neighboring creeks, we wanted to document the physical, chemical, and biologic nature of these two creeks.

Mud Creek and Rock Creek are located in the extreme southern portion of the Cascade Mountain province and lie predominantly in Butte County, California. Their watersheds drain portions of the western slope of the Cascade Mountains and flow west into the Sacramento Valley.

During the course of our investigations we made several interesting observations such as the capture of several large California roach (>100mm). This population may be a sub-species, only more extensive sampling will answer that question. We also identified a sub-terranean Amphipod, which may never have been recorded in this part of the country before. These watersheds may truly be unique in their species richness and the geology that dictates species distributions. Additional sampling will be conducted during summer and fall 1997 to accurately determine the composition, abundance, and distribution of aquatic populations with the goal that this information will be used to create better management of the area.

Gene expression patterns in *Pinus ponderosa* under elevated CO₂

David Garcia-Ibáñeta

Sponsored by Dr. James Pushnik

The concentration of atmospheric carbon dioxide is expected to double by the middle of the next century. As a result, the mean global temperature and precipitation patterns are expected to shift. Photosynthesis is a direct link between inorganic and organic carbon. Thus, it has become imperative that we begin to assess the potential impacts of a high CO₂ environment on our forest ecosystems. The purpose of this research is to identify specific CO₂-induced genetic expression patterns associated with enhanced photosynthesis and increased biomass accumulation in *Pinus ponderosa*. This information will become vital to the breeding or biotechnological improvement of reforestation efforts in our National Forests, as these trees will certainly be subjected to altered atmospheric composition.

This poster presents the strategy that is being employed to identify currently unknown CO₂ responsive genes using reverse transcriptase-polymerase chain reactions (RT PCR) amplified mRNA and Differential Gene Display methodology. Additionally, this technique is being tested as a possible route for the specific identification and amplification of known CO₂ responsive genes using sucrose-phosphate synthase as a model. Initial results of both approaches are quite encouraging.

Optimization of biodegradative rates for soluble petroleum hydrocarbons

Carina M. Jung

Sponsored by Dr. Larry Hanne and Alex Rafalovich of Metacalf & Eddy Inc.

Soil treatability studies have been conducted at Beale Air Force Base (AFB), CA since 1995 to develop economical methods for the ex-situ biodegradation of water soluble total petroleum hydrocarbon (s-TPH) contamination. Earlier studies showed that bio-catalytic additives (enzyme mixtures) degraded s-TPH at rates significantly higher than those observed with traditional landfarming soil amendments (inorganic fertilizer, compost, microbial inocula). Follow-up bench-scale testing was employed to develop an optimal economic formulation for the bio-catalytic additive and to assess the effect of the additive on the native microbial community. The results yielded a new bio-catalyst formulation that will be lower in price and will treat s-TPH in soil at degradation rates four times faster than previously observed for TPH-contaminated soil at Beale AFB. Populations of petroleum hydrocarbon degrading bacteria were determined to increase by an order of magnitude in those samples which had bio-catalytic additives versus those that received no bio-catalyst.

Comparison of Four Populations of California Roach (*Lavinia symmetricus*) using RAPD Markers.

Ronald Loggins

Sponsored by Dr. Jeff Bell

The California Roach (*Lavinia symmetricus*) is a small minnow found only in California. The roach is found almost exclusively in low-elevation foothill streams. The populations in these streams are often isolated from one another because the Roach is unable to persist in valley floor or large river habitats, especially in the presence of predatory fishes. The isolation of these populations is increasing because of intense agricultural development of lowland areas, damming of large rivers, and the introduction of predatory fishes into impoundments and larger streams.

This study assesses the genetic variation within and between four populations of Roach using RAPD markers. RAPD creates visible genomic markers by using arbitrary primers and the Polymerase Chain Reaction (PCR) on

DNA isolated from individual organisms. Comparisons between populations in Big Chico, Butte, Pine and Zimmershed Creeks were made by following four independent analysis procedures.

The four analysis techniques employed indicated that the four populations are genetically isolated from each other. Along with being isolated, each population is very small. This is indicated by the low intra-population heterozygosity and a high intra-population similarity. Finally, it appears that these populations of Roach have remained isolated for a long time. This conclusion is based on the high genetic distances between the populations.

Since this fish lives in a habitat which is being encroached upon by human activities, it is imperative to know as much about its biology and genetic character if we are to make effective decisions in managing each Roach population.

The effects of two fungicides, vinclozolin and iprodione, on the in utero development of reproductive structures in rats

Kelly M. Wood

Sponsored by Dr. Robert Thomas

A recent issue of public concern is whether commonly used pesticides are safe when delivered into the environment. Since the 1960s researchers have found various effects on humans and wildlife that were unintentionally exposed to pesticides.

One particular research experiment was published in 1994 and examined the effects of vinclozolin (a fungicide), on the male offspring of rats exposed in utero. It was found that the fungicide caused reproductive malformations including a reduced anal genital distance, cleft phallus, vaginal pouches, hypospadias, ectopic testes, and a decreased weight in the seminal vesicles and ventral prostates. Another fungicide, called Iprodione, is very similar in chemical structure to vinclozolin and has many of the same characteristics. It was felt that the two chemicals should therefore have similar reproductive effects.

In the experiment, 18 pregnant rats were used and divided into three groups. Each rat was dosed with 2% of their LD50 (200mg/kg/day of vinclozolin or 70mg/kg/day of iprodione), in 2.5ml/kg/day of corn oil (controls were given corn oil alone). The rats were dosed from 14 days gestation to 3 days lactation then the offspring were observed for malformations up to adulthood. Maternal information was noted, body measurements on the offspring were made, pubertal indices checked, and organ weights determined.

There was no significance found in body measurements or organ weights in the iprodione group and only a slight delay of puberty found in the female offspring and early onset of puberty in the males. The vinclozolin results replicated those reported in the literature. Iprodione although similar in structure, does not mimic the effects as vinclozolin on reproductive development