Oklahoma Water Research Institute
Annual Technical Report
FY 2003

Introduction

The Environmental Institute at Oklahoma State University has as its mission to serve as a center for stimulation and promotion of interdisciplinary research, graduate education and public education relating to understanding, protecting, utilizing and sustaining the natural environment. The federally supported Oklahoma Water Resources Research Institute, created under Section 104 of the Water Resources Research Act, is one of 54 Water Institutes. In Fiscal Year 2003, the $84,234 grant to OWRRI was matched by $174,338 in non-federal money. These funds supported three research projects and water research administration and development activities as well as the information transfer program. The three research projects supported by the OWRRI program are as follows: Project 2003OK28B Facilitating the Tenkiller Utilities Authority Public Water Decision Project develops and assesses the efficacy of a protocol for the facilitation of water distribution-related disputes. Project 2003OK17B Dual sensor for detecting xenobiotics and microorganisms is basic research supporting the development of a sensor to detect contaminants in drinking water. Project 2003OK16B Algal-nutrient dynamics in fresh waters: direct and indirect effects of zooplankton grazing and nutrient remineralization examine the role and magnitude of planktonic consumer-driven nutrient regeneration in mesotrophic Lake Texoma.

Research Program

Algal-nutrient dynamics in fresh waters: direct and indirect effects of zooplankton grazing and nutrient remineralization

Basic Information

Title:	Algal-nutrient dynamics in fresh waters: direct and indirect effects of zooplankton grazing and nutrient remineralization
Project Number:	2003OK16B
Start Date:	3/1/2003
End Date:	2/28/2004
Funding Source:	104B
Congressional District:	4th
Research Category:	Not Applicable
Focus Category:	Nutrients, Non Point Pollution, Surface Water
Descriptors:	
Principal Investigators:	K. David Hambright, K. David Hambright

Publication

SYNOPSIS

Project Number: _____ **Start date:** 01 March 2003

End date: 28 February 2004

Title:

Algal-nutrient dynamics in fresh waters: direct and indirect effects of zooplankton grazing and nutrient remineralization

Investigator:

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Congressional District:

4th Congressional District of Oklahoma

Descriptors:

nutrient-algal ecology, zooplankton, grazing, nutrient cycling, nutrient supply, water quality, freshwater reservoirs, Lake Texoma

Problem and Research Objectives:

Lake Texoma is a large (360 km^2) impoundment of the Red and Washita Rivers draining more than 100,000 km^2 in Oklahoma, Texas and New Mexico. Although Lake Texoma was designed primarily for flood control and hydropower generation, like many reservoirs throughout Oklahoma, water supply and tourism (including fishing) have become primary year-round uses for the reservoir. As such, water quality is a major focus of lake management. With respect to quality of water supply, chloride concentrations receive much emphasis, however, nutrient (especially phosphorus and nitrogen) loading and concentrations are also critical factors affecting water quality for both water supply and tourism. High nutrient loading from the water shed yields relatively high productivity, especially in mid-summer, with potentially nuisance cyanobacteria (bluegreen algae) *Aphanizomenon*, *Anabaena* and *Microcystis* dominating the phytoplankton (algal) assemblage. With most nutrient sources today being non-point sources (e.g., agricultural runoff) that are difficult to regulate, management of internal lake nutrient cycling via food web management may be a viable alternative for regulating water quality. In many lakes worldwide, managers attempt to manipulate lake food webs toward systems in which phytoplankton are suppressed by intensified grazing and reduced rates of nutrient cycling by zooplankton (Drenner and Hambright 1999).

The objective of this research was to examine the role and magnitude of planktonic consumer-driven nutrient regeneration in mesotrophic Lake Texoma. Using laboratory mesocosm experiments based on consumer-food encounter rate models, I attempted to quantify

grazing rates and nutrient remineralization rates by both macro- and micro-zooplankton assemblages.

Methodology:

The basic design employed for determining zooplankton grazing and nutrient recycling rates consists of measuring changes in abundances of bacteria and algae and in concentrations of dissolved nutrients over a 24-h period in experimental mesocosms containing a gradient of plankton densities, and hence a gradient of consumer-food encounter rates (Lehman 1980a, b, Landry and Hassett 1982).

A benefit of this approach is that the confounding effects of simultaneous uptake of nutrients by bacteria and algae are absent. By adding excess nutrients at the beginning of an experiment, rates of nutrient uptake by bacteria and algae are constant and independent of nutrient recycling by the zooplankton during the short duration of the experiment (24 h). Hence growth rates of bacteria and algae are not positively affected by density-dependent zooplankton nutrient excretion. Zooplankton grazing rates, zooplankton nutrient excretion rates, and maximum potential nutrient uptake rates by bacteria and algae (in the absence of zooplankton) can then be determined from the changes in abundances of bacteria, algae, and nutrient concentrations as functions of zooplankton biomass. Experiments can be performed at different times of the year to provide insight into seasonal variability in grazing and nutrient remineralization.

Grazing: Theoretical Framework of Approach

During an experiment, biomass of bacteria or algae A can potentially change as

$$dA / dt = r_A A \qquad (1)$$

where rA is the net rate of change in biomass due to grazing mortality g and reproduction or growth k, (i.e., $r_A = k - g$) and is calculated as

$$r_A = (lnA_t - lnA_0) / \Delta t \qquad (2)$$

where A_t and A_0 are biomasses of bacteria or algae present at the end and beginning of the experiment ; Δt is 1 day. No net change is indicated by $r_A = 0$; a net increase by $r_A > 0$, and a net decrease by $r_A < 0$. According to the Lehman model, grazing rates can be quantified by linear regression of r_A on zooplankton biomass, Z (Figure 1).

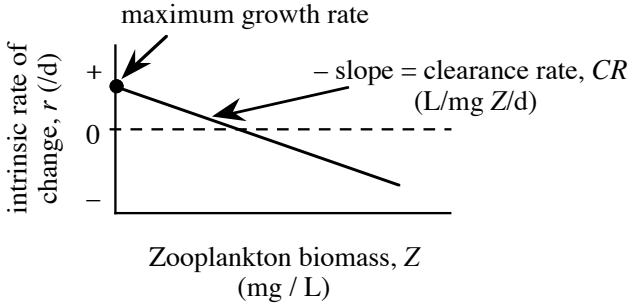

Figure 1. Demonstration of the relationship between bacterial or algal rate of change, r, and zooplankton biomass, Z, showing estimation of clearance rate, CR, (the amount of water cleared of bacteria or algae per biomass of zooplankton per day) and maximum growth rate of the bacteria or phytoplankton at saturating nutrient concentrations in the absence of grazing.

Because nutrients are added at the beginning of an experiment at algal and bacterial growth-saturating concentrations, enhanced growth is expected. The y-intercept of this regression, where zooplankton biomass = 0, indicates the maximum rate of growth for the bacteria or algae *A* in the absence of grazing and has units of per day. The slope of this regression indicates zooplankton-dependent effects; its negative equals the clearance rate (*CR*) in units of L Z^{-1} d^{-1}. A slope of 0 indicates that the bacteria or algae under consideration was not grazed.
Grazing rates, *GR*, can be calculated as the product of the mean biomass of the bacteria or algae and the clearance rate ($GR = CR * A$), where *A*, the mean biomass of bacteria or algae is calculated as

$$A = (A_0 - A_t)\ [(r_A)\ (\Delta t)]^{-1}. \qquad (3)$$

The basic assumptions of the Landry-Hassett model are similar in nature to those of the Lehman model. The principle difference between the two models is the manner in which the encounter-rate dependency of the grazer-food (i.e., zooplankton-algae and bacteria) relationship is manipulated although both manipulations yield the same result. In the Lehman model, the grazer densities are manipulated directly by adding increasing amounts of macro-zooplankton to a series of experimental bottles; in the Landry-Hassett model, both grazer and food densities are manipulated by diluting whole lake water (containing ambient densities of grazers and food) with lake water in which all grazers and food have been removed by filtration. According to the Landry-Hassett model, the probability of a food item being grazed is a direct function of the rate of encounter of grazers with food items and that encounter rates are directly proportional to grazer and food densities, thus $r_A = k - g$ becomes

$$r_A = k - Xg, \qquad (4)$$

where *X* is the dilution factor (i.e., the fraction of non-filtered water in the experimental bottles). Therefore, any observed rates of change in food taxa during an experiment (Eq. 1) at different dilutions are linearly related to the dilution factor (Figure 2).

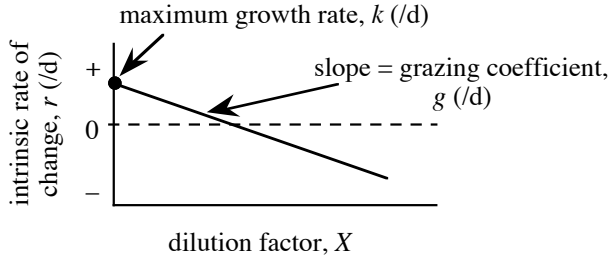

Figure 2. Demonstration of the relationship between phytoplankton rate of change, *r*, and the dilution factor (ratio of unfiltered to filtered lake water) allowing calculation of the grazing coefficient, *g*, where $r = k - Xg$. The maximum growth rate of the phytoplankton at saturating nutrient concentrations in the absence of grazing, *k*, is estimated as the y-intercept.

Nutrient Remineralization: Theoretical Framework of Approach.

During an experiment, changes in nutrient concentrations can be described as

$$dS/dt = -uA + cZ \qquad (5)$$

where *S* is the nutrient concentration, *A* is the biomass of food taxa (i.e., algae and bacteria), *Z* is the biomass of zooplankton estimated by direct counts, u is the rate of nutrient uptake by the phytoplankton and bacteria, and c is the rate of nutrient recycling by the zooplankton (Lehman 1980a, b, Landry-Hassett 1982). Because nutrients are added at the beginning of the experiment at saturating conditions such that $du / dS = 0$, equation (5) can be integrated over a time period of 1 day and rewritten as

$$-\Delta S / A = - cZ / A + u. \qquad (6)$$

Both *c* and *u* can then be computed by linear regression of $-\Delta S / A$ on Z / A (Figure 3).

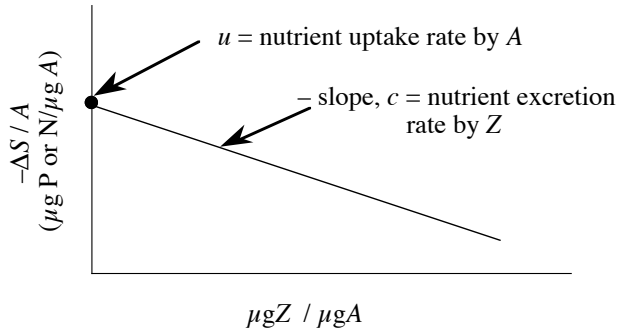

Figure 3. Demonstration of the relationship between changes in nutrient concentrations over 24 hrs as a function of zooplankton biomass. The maximum nutrient uptake rate, u, for the phytoplankton (or other food taxon), A, and the excretion rate of that nutrient by the grazing zooplankton, c, are calculated when both the change in nutrient concentrations and zooplankton biomass are standardized to the biomass of phytoplankton (or other food taxon).

The negative of the slope of this regression equals the rate at which a nutrient is excreted by zooplankton. The intercept of this regression equals the maximum potential rate of nutrient uptake by the phytoplankton and bacteria. Because of the initial nutrient-saturated conditions, it could be considered that the obtained values of excretion may be maximum estimates. However, results of radio-isotope tracer experiments (Hambright et al. in prep.) confirmed, at least for P, that such estimates of excretion by this method are realistic.

Experimental Protocol: Macro-zooplankton:

Macro-zooplankton grazing and nutrient recycling rates were measured using an experimental protocol according to Lehman (1980a, b) and as determined in my previous research with Lake Kinneret plankton assemblages (Hambright et al. 2001a, b). The experiments were conducted in 10-L, clear polystyrene bottles filled with lake water (from 5m depth at a central lake station) filtered through 150 μm-mesh netting (to remove macro-zooplankton but retain the natural assemblage of algae and bacteria). Bottles were placed on a laboratory bottle roller (apparatus for maintaining optimal light and preventing settling of suspended particles, including algae) inside a large walk-in growth chamber that allowed for maintenance of natural light and thermal regimes for a given season. Four bottles were stocked with zooplankton at naturally-occurring densities; four with 2X naturally-occurring densities; four with 4X naturally-occurring densities; and four remained without macro-zooplankton. All 16 bottles were enriched with inorganic nitrogen (500 μg N as NH_4Cl) and phosphorus (50μg P as Na_2HPO_4),

concentrations sufficient for saturating algal and bacterial uptake rates (Hambright et al. 2001a). All bottles were sampled at 0 and 24h to determine initial and final concentrations of size-fractionated chlorophyll *a* , NH_4^+ , SRP-PO_4^{-3}, total nitrogen (TN) and total phosphorus (TP), zooplankton, phytoplankton, bacteria and protists as described below. Because other N and P sources can be important seasonally, I also monitored initial and final concentrations of NO_3^- and total dissolved P (TDP).

These data were analyzed as functions of zooplankton biomass according to Lehman (1980a, b) to assess grazing and nutrient remineralization rates of macro-zooplankton. Because micro-zooplankton were also present in the bottles, the final calculated rates of grazing and nutrient remineralization were corrected to account for effects of micro-zooplankton using results from paired micro-zooplankton experiments described in the following section.

Experimental Protocol: Micro-zooplankton:

Micro-zooplankton grazing and nutrient recycling rates were measured using an experimental protocol according to Landry and Hassett (1982). Experiments were conducted in 2-L polystyrene bottles maintained in the walk-in growth chamber as detailed above for macro-zooplankton. Forty liters of lake water were collected from a central lake station, filtered through 150 μm mesh to remove macro-zooplankton and returned to the laboratory. Half of this water was filtered through 0.2μm mesh to remove all plankton, including algae and bacteria (checked microscopically). This filtered lake water was combined with the remaining non-filtered lake water in ratios of unfiltered to filtered water of 1:0 (100% unfiltered), 3:1 (75%), 1:1 (50%), and 1:3 (25%). Four 2-L bottles were filled with each dilution mixture and excess N and P added to each bottle similar to the paired experiment for macro-zooplankton describe above. All 16 bottles were sampled as in the macro-zooplankton experiments, with data analyzed as functions of the dilution mixture according to Landry and Hassett (1982) to assess grazing and nutrient remineralization rates for micro-zooplankton. Additionally, these resultant micro-zooplankton grazing and nutrient remineralization rates were used to "correct" the rates calculated for the paired macro-zooplankton experiment (i.e., micro-zooplankton effects were subtracted from the combined effects of macro- and micro-zooplankton in the 10-L mesocosms).

Sample analyses

Chlorophyll: Chlorophyll concentrations were determined fluorometrically on whole and filtered (2, 25 μm) water following methanol-chloroform extraction (Wood 1985). Net-chlorophyll was calculated by subtraction of the <25 μm fraction from whole-water chlorophyll; nano-chlorophyll by subtraction of the <2 μm from the <25μm fraction; pico-chlorophyll as the <2 μm fraction. Chlorophyll values were converted to carbon assuming C:chl = 50 (Hambright et al. 2001a).

Phytoplankton: Phytoplankton were preserved in Lugol's iodine solution and examined microscopically to determine dominant taxa in each algal size class.

Bacteria: Bacteria were preserved in 10% filtered (0.45μm) Formalin, stained using DAPI and enumerated and measured using epifluorescent microscopy and Image-Pro software. Densities and biovolumes were converted to carbon according to Simon and Azam (1989).

Zooplankton: Zooplankton biomass was determined by direct microscopical counts of ethanol-preserved samples taken from the beginning and end of each experiment followed by conversion to carbon according to Culver et al. (1985) and Hambright et al. (2001a).

Protozoans: Ciliated-protozoans were enumerated on 5X concentrated (by sedimentation), Lugol's-preserved samples using an inverted microscope. Flagellated protozoans were enumerated with epifluorescent microscopy following preservation in 10% filtered Formalin and staining using DAPI. Densities and biovolumes were converted to carbon according to Putt and Stockner (1989).

Nutrients: Nutrients were analyzed following persulfate digestion (at 100°C for 1 hour) of whole water (total phosphorus and total nitrogen) or filtered (0.2 μm) water (total dissolved phosphorus and nitrate). Soluble reactive phosphate and ammonia was analyzed on filtered (0.2 μm) water. All procedures were according to standard methods (APHA 1995).

Statistical analyses: Zooplankton grazing and nutrient remineralization rates were examined using linear regression (by least squares) of the relationships detailed in Figs. 1, 2, and 3. Significance for the null hypothesis that the slope of the regression is equal to or greater than zero (H_0: slope ≥ 0) was set at $\alpha = 0.05$.

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Principal Findings and Significance:

In this project, I set out to experimentally quantify zooplankton grazing and nutrient remineralization rates in Lake Texoma. Initially, I planned to conduct two experimental series during the year, each series including macro- and micro-zooplankton grazing experiments run in tandem. An additional experimental series was made possible through an OU-UROP grant to an undergraduate student working in my lab. Each experiment, lasting only 24 hours, required extensive set-up and preparation, including collection and sorting of zooplankton, calibration of methodology in accordance with ambient plankton and nutrient concentrations and actual preparation of experimental treatments. Following 3 months of preliminary testing during summer 2003, six experimental series were attempted, two of which were aborted before completion, leaving four series run through completion. In none of the four complete series of experiments did we detect significant grazing by either macro- or micro-zooplankton. Most of these findings can be attributed to either insufficient densities of zooplankton in the mesocosms or failure of the mesocosm roller unit (Fig. 4). After extensive delay in obtaining appropriate

Figure 4. UOBS bottle roller, showing 16 macrozooplankton (12-L bottles; bottom) and 12 microzooplankton (2.5-L bottles; top) mesocosms. Bottles rotate at 1-4 revolutions per minute and change directions every 5 minutes to prevent sedimentation and aggregation of planktonic particles. Ambient temperature and light are maintained to mimic lake conditions of interest.

parts (mostly relating to electronic controls regulating rotation speed, direction and timing), the mesocosm roller is now in working condition, and should therefore not be an issue with future experiments. Likewise, after more than a year of working with Texoma plankton in the mesocosm facility, I am better able to judge appropriate densities conducive to successful experiments. Thus, although this project is officially finished, I will continue to pursue this issue of zooplankton grazing and nutrient remineralization in Lake Texoma.

Indeed, looking to the future, a few successes can be noted for this project. First, all equipment necessary for successful completion of grazing experiments are now available in my laboratory and will therefore contribute to ongoing and future research in zooplankton ecology in Lake Texoma. Second, two students received direct, hands-on training in project methodologies and learned valuable lessons with respect to experimental design and the potentially low success rates in experimentation with living animal and plant assemblages. The first student, a 3rd-year undergraduate at OU, has decided to pursue graduate-level education in limnology, with emphasis relating to water-quality research. The other student is currently pursuing his PhD (2nd year) at OU in my laboratory.

Thus, while I have so far been unable to obtain many of the specified project objectives, I am confident that the ground work laid during this project will serve great impetus in ongoing and toward future experimental analyses of zooplankton grazing and nutrient remineralization, both in my research and in that of my students.

Awards

None

Grants obtained from this Grant

Ms. Nicole Luke (OU undergraduate 3rd year) received a $500 OU Honors College UROP (Undergraduate Research Opportunities Program) grant entitled "Grazing and nutrient remineralization in *Daphnia lumholtzi*" to support her independent research related to this project. She will be presenting her research findings at the OU Undergraduate Research Day in April 2005.

PUBLICATIONS

There are no current publications relating to this project.

STUDENTS SUPPORTED BY THIS PROJECT

	Number	Discipline
Undergraduates:	1	Zoology
Masters		
Ph.D.	1	Ecology & Evolutionary Biology
Post Doc		
Total	2	

Dual sensor for detecting xenobiotics and microorganisms

Basic Information

Title:	Dual sensor for detecting xenobiotics and microorganisms
Project Number:	2003OK17B
Start Date:	3/1/2003
End Date:	2/28/2004
Funding Source:	104B
Congressional District:	3rd
Research Category:	None
Focus Category:	Surface Water, Toxic Substances, Water Quality
Descriptors:	None
Principal Investigators:	Gilbert John, Mario Rivera, Gary Yen

Publication

A. INTERIM ANNUAL REPORT

Project ID: 2003OK17B

Title: Dual sensor for detecting xenobiotics and microorganisms

Project Type: Research

Focus Categories: Surface Water, Toxic Substances, Water Quality

Keywords: spectrophotometer, cytochrome P450, autofluorescence

Start Date: 03/01/2003

End Date: 02/28/2004

Federal Funds Requested: $25000.00

Matching Funds: $50000.00

Congressional District: 3rd

Principal Investigators: John, Gilbert; Rivera, Mario; Yen, Gary

Oklahoma Water Resources Research Institute (OWRRI)
July 2004

B. RESEARCH

INTRODUCTION:

Since September 11th, Homeland Security in the United States has become more important, as many aspects of security in this country are being examined and developed. One aspect is the security of drinking water. Deliberate contamination of drinking water make it imperative to have an efficient, sensitive, specific and rapid sensor that can detect both xenobiotics and microbial organisms that can cause harm to individuals. Billions of dollars are being made available from government and state agencies to develop systems that can continuously monitor drinking water. A multi-discipline group at Oklahoma State University is involved in developing a dual sensor that can be used in this capacity. Our proposal specifically addresses two critical areas that are important for further development of a dual sensor that can detect potentially harmful xenobiotics (toxicants) and pathogenic bacteria in water. The first area specifically addresses the issue of having stable proteins that can maintain their function under various environmental conditions. The cytochrome (CYP) P450 protein from the human liver is normally involved in

detoxifying and toxifying a broad range of xenobiotics, thereby CYP proteins can be used to directly link xenobiotics to human toxicity. A number of isoforms are present in the liver, but some of these proteins are not stable (CYP3A4), compared to stable proteins (CYP1A2). Therefore, the first area we addressed was to develop a method of improving stability of CYP 3A4 using molecular modeling techniques thereby increasing ion-pair interactions in the protein. The second area addressed was to examine the autofluorescence signatures (spectrofluorimetry) from bacteria, which may provide a means of identifying different types of bacterial pathogens. Available methods that can be used to improve the stability of cytochrome P450 without compromising function as well as having unique spectra that can be used to specifically identify potentially harmful pathogens is critical for future development of a dual sensor.

The research report addresses two areas, 1.) development of a computer graphics method for improving protein stability, which will enable the proteins to effectively detect potentially harmful xenobiotics and 2.) to determine if autofluorescence signatures from whole bacteria can be used to detect pathogenic bacteria in water.

METHODS AND RESULTS

Area 1.) To improve the stability of cytochrome P450 proteins for use in detecting xenobiotics, a computer graphics-modeling program was necessary in order to determine the important residues involved in protein stability. Three graphics programs were identified to have functions that were relevant and applicable to the project. They included PyMOL (Delano Scientific, http://www.delanoscientific.com), DeepView(Swiss Model), and WHAT IF Web Interface). Computer modeling coordinates for CYP 1A2 and 3A4 were used to generate the protein models and were obtained from Dr. Lewis (United Kingdom). The mutant protein model was tested for distances (residue positions based on 3-D images) using PyMOL prior to submitting the model for residue alteration by the WHAT IF server. Since the computer model of CYP 1A2 and 3A4 were similar, the mutational prediction for 3A4 was possible based on the analysis of the hydrogen bonds networks and ion pair networks of the CYP 1A2. Mutations were predicted based the method of Chinea.G & Vriend.G, for position-specific rotamers (14). The WHAT IF web interface calculates distances in angstroms (15).

The computational analysis allowed the selection of five candidates for site-directed mutagenesis of the CYP 3A4. The candidates were selected based on the number of additional ion pairs, hydrogen bonds, and additional residue interactions that were created based on the model and data received from the WHAT IF web interface.

Different stabilities exist within the family of cytochromes (CYP). Therefore, we hypothesize that some of the stability is due to ion pairs and/or ion networks (1-7). WHAT IF (http://swift.cmbi.kun.nl/WIWWWI/), a web server, was used to locate the number of optimal hydrogen bonds networks and salt bridge locations within the proteins according to the protein data bank files. Using the computer graphics programs, it was shown that the wildtype CYP 1A2 (more stable) had more ion pair networks than CYP 3A4 (less stable). To improve CYP 3A4 stability, residues distant from the substrate-binding region were selected for site-directed mutagenesis. Figure 1 shows the five selection residues selected for mutation, based on the superimposition generated by PyMOL. Figure 2 shows the GLU66=>ASP66 change, which increases the salts bridges (ion pairs) from 2 to 3, as well as providing a supporting hydrogen bond network (Table

1 and 2). Figure 3 shows the VAL124=>LYS124 change, which increases the salt bridges from 0 to 4 and maintains supporting hydrogen bond networks (Table 3 and 4). Figure 4 shows the GLY146=>ARG146 change, which increases the salt bridges from 0 11 and maintains supporting hydrogen bond networks (Table 5 and 6). Figure 5 shows the TYR376=>HIS376 change, which increases the salt bridges from 0 to 5 and maintains supporting hydrogen bond networks (Table 7 and 8). Figure 6 shows the ASN431=>ASP431 change, which increases the salt bridges from 0 to 3 and maintains supporting hydrogen bond networks (Table 9 and 10). Incorporation of all or selected mutations will be tested.

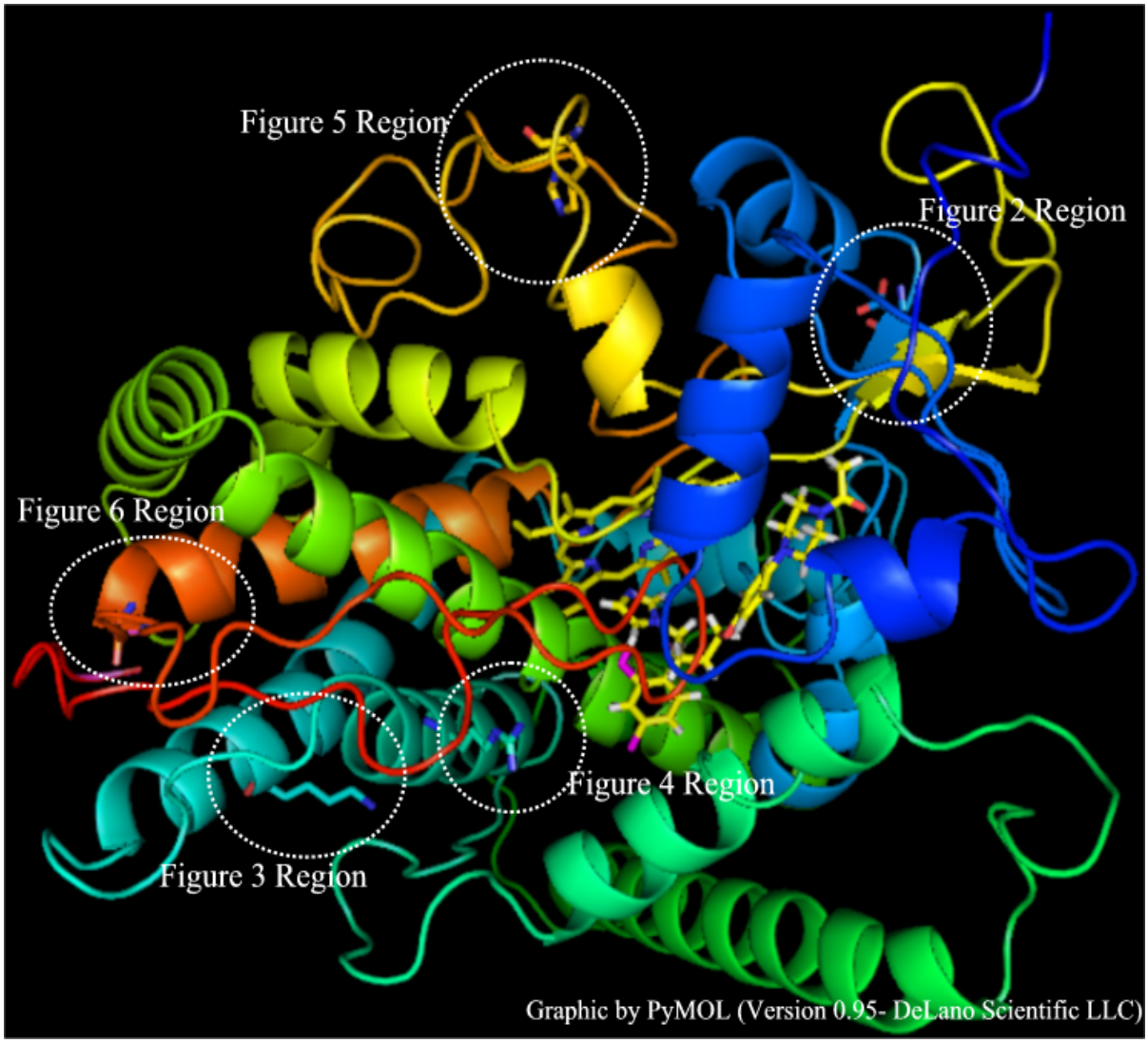

Figure 1: Superimposition of wild-type (wt) CYP3A4 and Mutant (m3a4) illustrating entire enzyme structure*.

a. Each ellipse corresponds to a mutant containing region.
b. Mutant residues are seen above as sticks.

* Graphic by PyMOL DeLano Scientific LLC & Edited with Microsoft PhotoDraw for all figures.

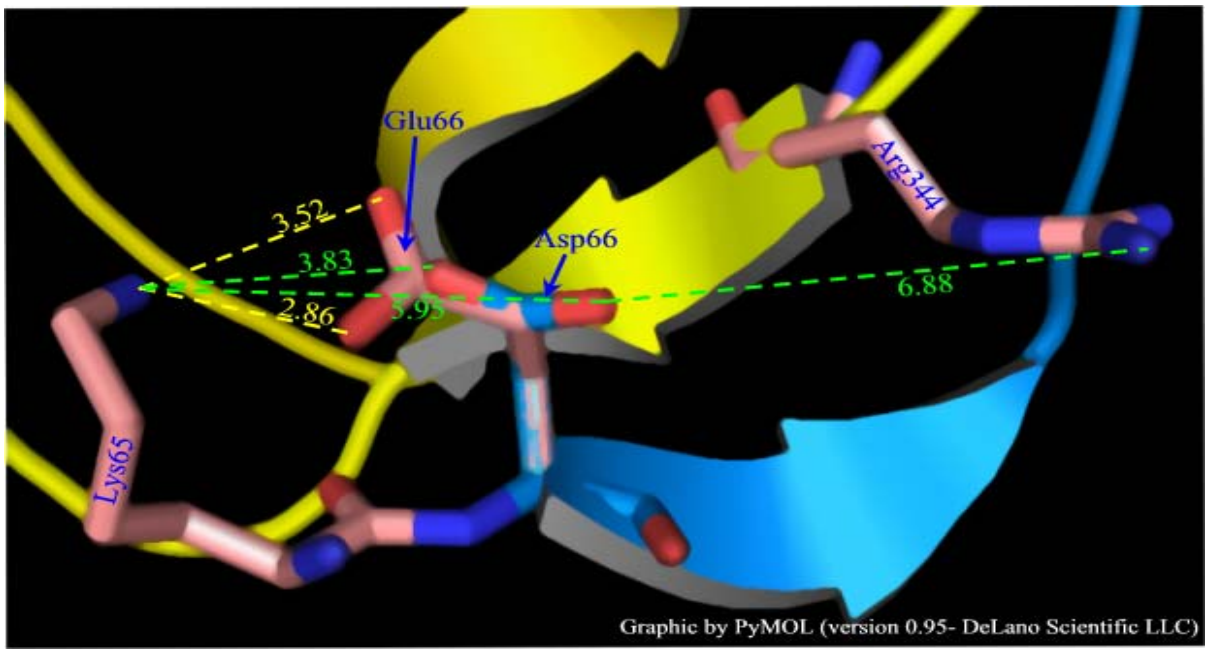

Fig. 2: Superimposition of wt- Glu66** and Mutant- Asp66 (m66)

a. m66 shown in blue sticks entangled with Glu66 pink
b. Ionic interaction- wt in yellow and m66 in green
c. Ionic interaction distance is in Å.
d. Residues Lys60, Thr61, Val62, Leu63, & Val64 were removed for clarity.

Table 1: WHAT IF- Salt Bridge Data (SBD), mutant data in boldface

Donor Residue	Donor Molecule	Acceptor Residue	Acceptor Molecule	Distance (Å)
66 ASP (66)	**OD1**	**65 LYS (65)**	**NZ**	**5.95**
66 ASP (66)	**OD2**	**65 LYS (65)**	**NZ**	**3.83**
66 ASP (66)	**OD1**	**344 ARG (344)**	**NH2**	**6.88**
66 GLU (66)	OE1	65 LYS (65)	NZ	3.52
66 GLU (66)	OE2	65 LYS (65)	NZ	2.86

Table 2: WHAT IF- Optimal Hydrogen Bond Network Data (OHBD), mutant data in boldface

Donor Residue	Donor Molecule	Acceptor Residue	Acceptor Molecule	Hydrogen Bond Value (0.0-1.0)[a]	Distance (Å)
65 LYS (65)	**NZ**	**-> 66 ASP (66)**	**OD2**	**Val= 0.437**	**DA= 3.83**
65 LYS (65)	N	-> 66 GLU (66)	OE2	Val= 0.390	DA= 2.85
65 LYS (65)	NZ	-> 66 GLU (66)	OE2	Val= 0.653	DA= 2.86

a. estimated importance of hydrogen bonds relative to each other. Perfect hydrogen bond = 1.0.

** Residue numbers based on coordinate file data for sequence residue numbers add thirty-two (32) for all data.

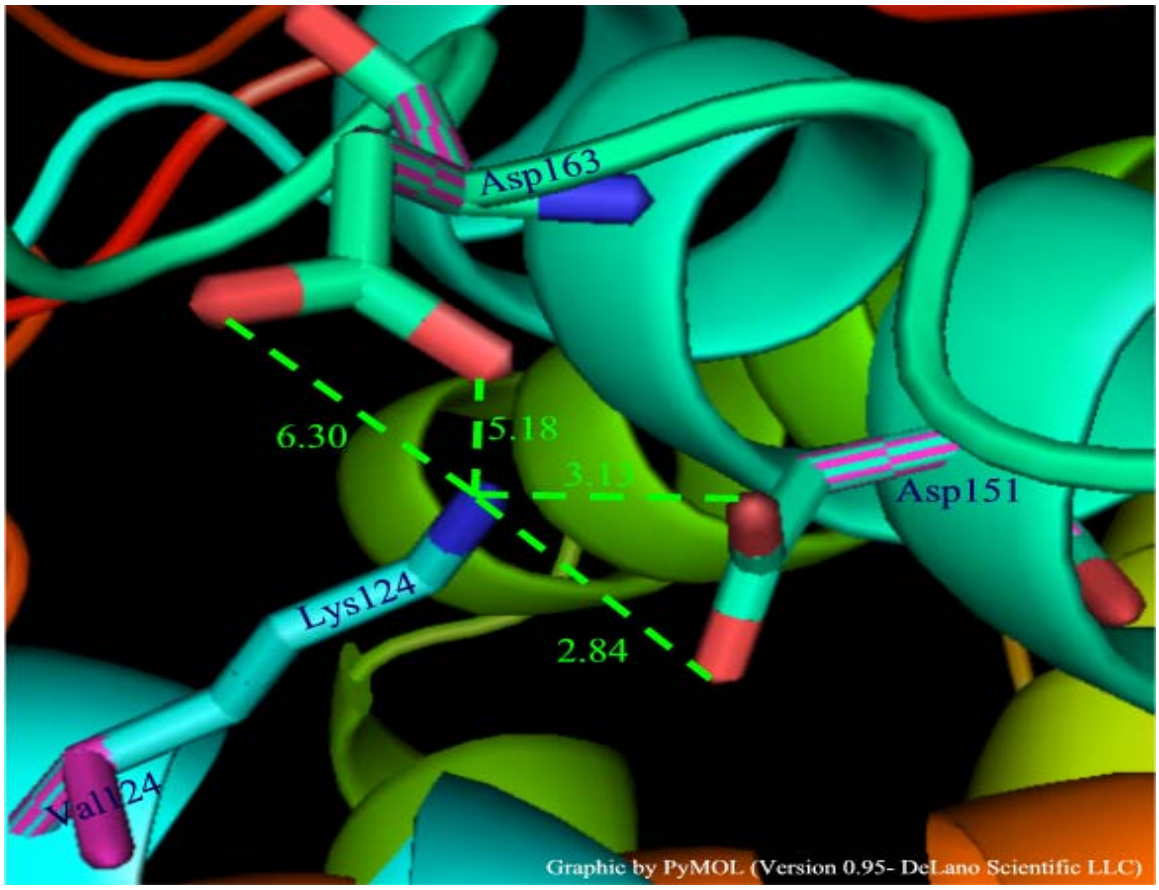

Figure 3: Superimposition of wt-Val124 and Mutant- Lys124 (m124)

a. m124 shown through Val124 striped, other stripes show superimposed residues.
b. Ionic interaction- wt-null and m124 in green
c. Ionic Interaction distance (green) in Å.

Table 3: WHAT IF- SBD

Donor Residue	Donor Molecule	Acceptor Residue	Acceptor Molecule	Distance (Å)
151 ASP (151)	**OD1**	**124 LYS (124)**	**NZ**	**3.13**
151 ASP (151)	**OD2**	**124 LYS (124)**	**NZ**	**2.84**
163 ASP (163)	**OD1**	**124 LYS (124)**	**NZ**	**5.18**
163 ASP (163)	**OD2**	**124 LYS (124)**	**NZ**	**6.30**

Table 4: WHAT IF - OHBN

Donor Residue	Donor Molecule	Acceptor Residue	Acceptor Molecule	Hydrogen Bond Value (0.0-1.0)[a]	Distance (Å)
124 LYS (124)	**N ->**	**120 GLN (120)**	**O**	**Val= 0.706**	**DA= 2.99**
128 ASN (128)	**N ->**	**124 LYS (124)**	**O**	**Val= 0.607**	**DA= 2.82**
124 VAL (124)	N ->	120 GLN (120)	O	Val= 0.706	DA= 2.99
128 ASN (128)	N ->	124 VAL (124)	O	Val= 0.607	DA= 2.82

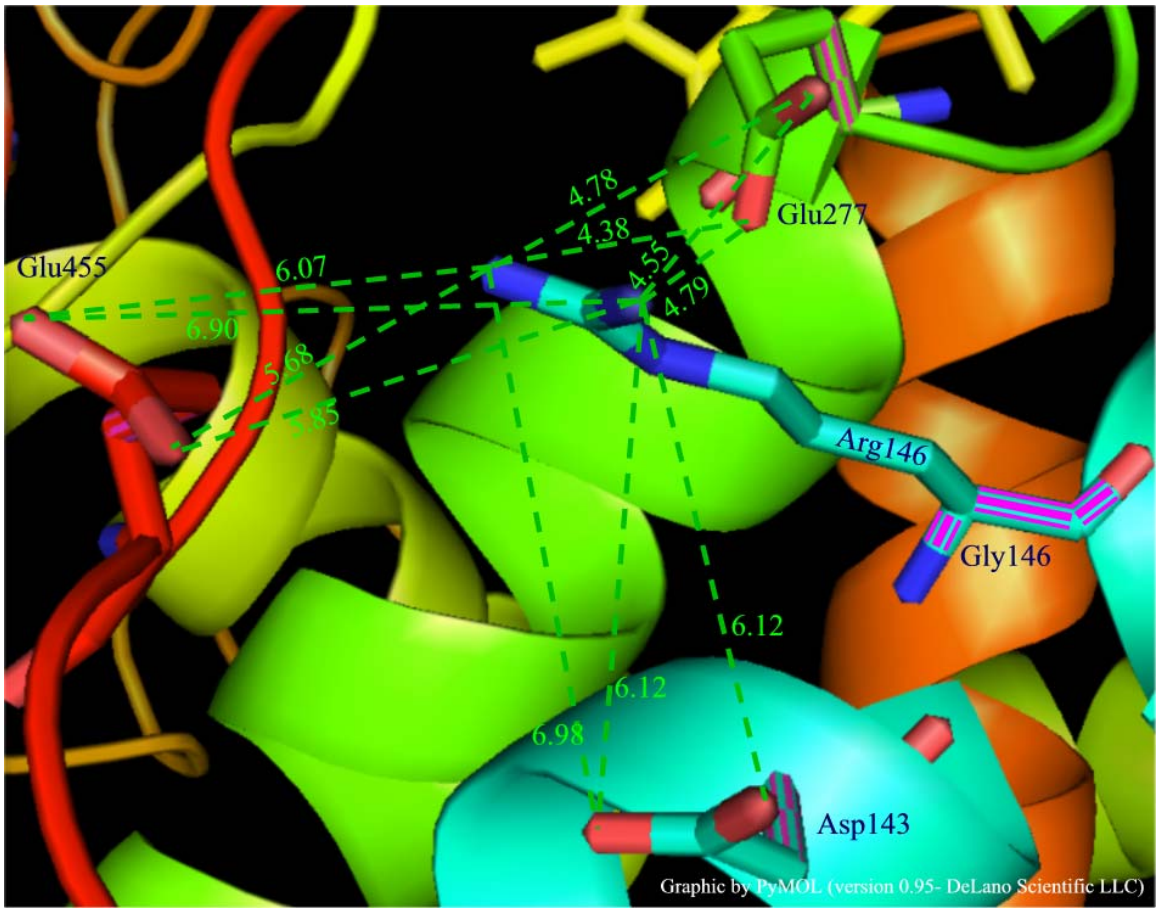

Figure 4: Superimposition of wt-Gly146 and Mutant- Arg146 (m146)

a. m146 shown through Gly146 striped, other stripes show superimposed residues.
b. Ionic interaction- wt-null and m146 in green
c. Ionic interaction distance (green) in Å.
d. Cartoon of main chain for wt and m146 has been removed at residue 146 for clarity.

Table 5: WHAT IF- SBD

Donor Residue	Donor Molecule	Acceptor Residue	Acceptor Molecule	Distance (Å)
143 ASP (143)	**OD1**	**146 ARG (146)**	**NH1**	**6.12**
143 ASP (143)	**OD1**	**146 ARG (146)**	**NH2**	**6.98**
143 ASP (143)	**OD2**	**146 ARG (146)**	**NH1**	**6.12**
277 GLU (277)	**OE1**	**146 ARG (146)**	**NH1**	**4.55**
277 GLU (277)	**OE1**	**146 ARG (146)**	**NH2**	**4.78**
277 GLU (277)	**OE2**	**146 ARG (146)**	**NH1**	**4.79**
277 GLU (277)	**OE2**	**146 ARG (146)**	**NH2**	**4.38**
455 GLU (455)	**OE1**	**146 ARG (146)**	**NH1**	**6.90**
455 GLU (455)	**OE1**	**146 ARG (146)**	**NH2**	**6.07**
455 GLU (455)	**OE2**	**146 ARG (146)**	**NH1**	**5.85**
455 GLU (455)	**OE2**	**146 ARG (146)**	**NH2**	**5.68**

Table 6: WHAT IF- OHBN

Donor Residue	Donor Molecule	Acceptor Residue	Acceptor Molecule	Hydrogen Bond Value (0.0-1.0)[a]	Distance (Å)
146 ARG (146)	**N ->**	**142 LYS (142)**	**O**	**Val= 0.629**	**DA= 3.19**
146 ARG (146)	**N ->**	**143 ASP (143)**	**O**	**Val= 0.090**	**DA= 3.04**
150 MET (150)	**N ->**	**146 ARG (146)**	**O**	**Val= 0.743**	**DA= 3.06**
146 GLY (146)	N ->	142 LYS (142)	O	Val= 0.629	DA= 3.19
146 GLY (146)	N ->	143 ASP (143)	O	Val= 0.090	DA= 3.04
150 MET (150)	N ->	146 GLY (146)	O	Val= 0.743	DA= 3.06

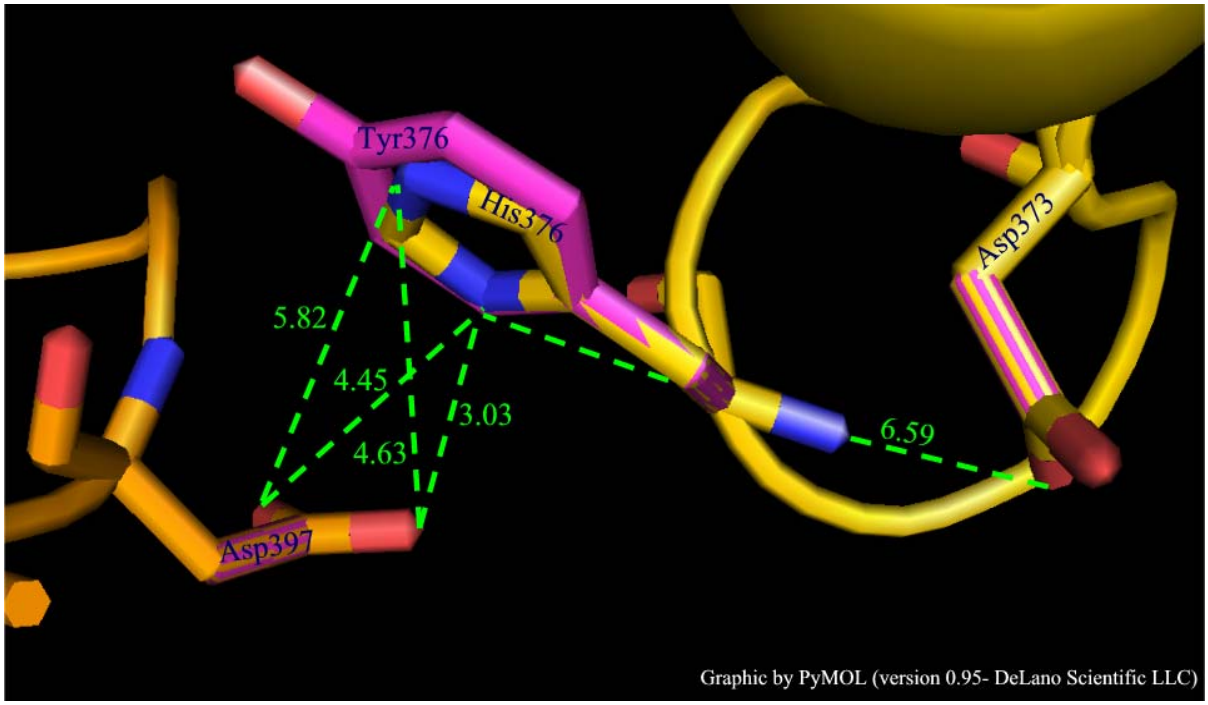

Figure 5: Superimposition of wt- Tyr376 and Mutant- His 376 (m376)

a. m376 shown entangled inside Tyr376 purple, other stripes show superimposed residues.
b. Ionic interaction- wt-null and m376 in green
c. Ionic interaction distance (green) in Å.
d. Residues Ile 365 & 400 removed for clarity.

Table 7: WHAT IF- SBD

Donor Residue	Donor Molecule	Acceptor Residue	Acceptor Molecule	Distance (Å)
373 ASP (373)	**OD1**	**376 HIS (376)**	**ND1**	**6.59**
397 ASP (397)	**OD1**	**376 HIS (376)**	**ND1**	**4.45**
397 ASP (397)	**OD1**	**376 HIS (376)**	**NE2**	**5.82**
397 ASP (397)	**OD2**	**376 HIS (376)**	**ND1**	**3.03**
397 ASP (397)	**OD2**	**376 HIS (376)**	**NE2**	**4.63**

Table 8: WHAT IF- OHBN

Donor Residue	Donor Molecule	Acceptor Residue	Acceptor Molecule	Hydrogen Bond Value (0.0-1.0)[a]	Distance (Å)
376 HIS (376)	**N ->**	**373 ASP (373)**	**OD1**	**Val= 0.666**	**DA= 3.19**
376 HIS (376)	**ND1 ->**	**397 ASP (397)**	**OD2**	**Val= 0.233**	**DA= 3.03**
376 TYR (376)	N ->	373 ASP (373)	OD1	Val= 0.666	DA= 3.19
376 TYR (376)	OH ->	397 ASP (397)	O	Val= 0.354	DA= 3.42

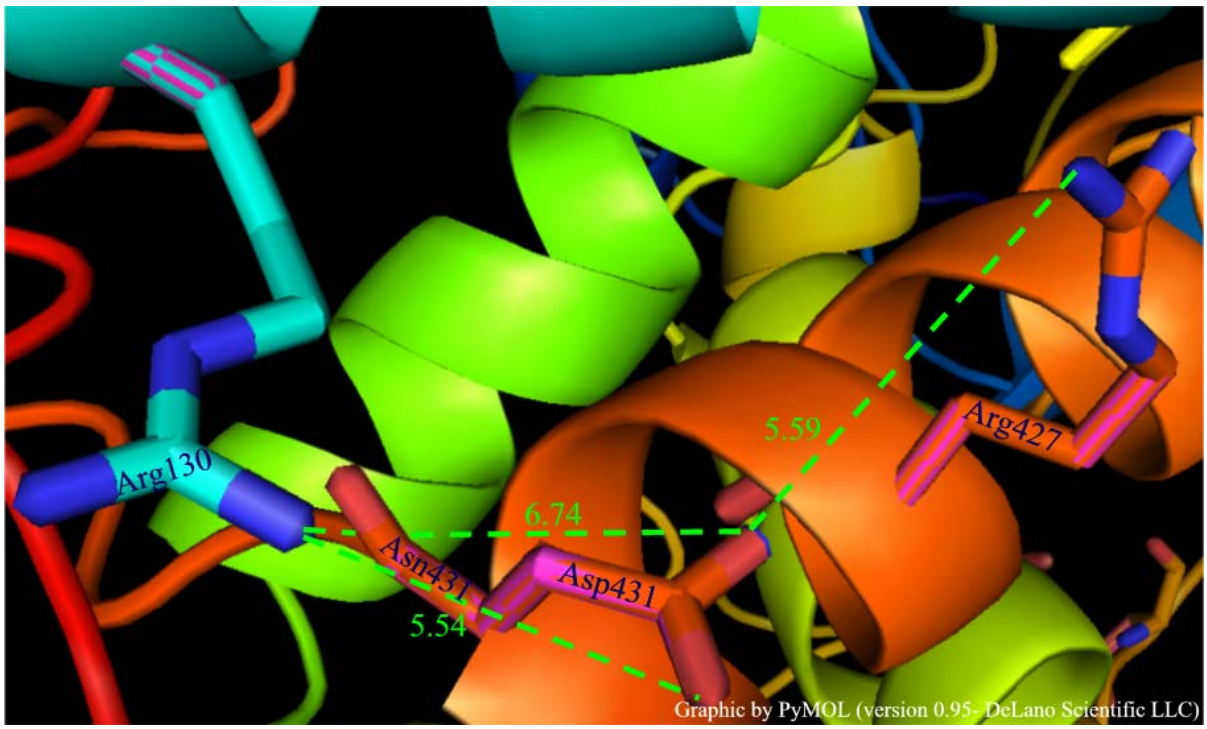

Figure 6: Superimposition of wt- Asn431 and Mutant- Asp431 (m431)

a. m431 red shown entangled inside Asn431 purple, other stripes show superimposed residues.
b. Ionic interaction- wt-null and m431 in green
c. Ionic interaction distance (green) in Å.
d. Residues Thr468 &Val469 removed for clarity.

Table 9: WHAT IF- SBD

Donor Residue	Donor Molecule	Acceptor Residue	Acceptor Molecule	Distance (Å)
431 ASP (431)	**OD1**	**130 ARG (130)**	**NH1**	**6.74**
431 ASP (431)	**OD2**	**130 ARG (130)**	**NH1**	**5.54**
431 ASP (431)	**OD1**	**427 ARG (427)**	**NH2**	**5.59**

Table 10: WHAT IF- OHBN

Donor Residue	Donor Molecule	Acceptor Residue	Acceptor Molecule	Hydrogen Bond Value (0.0-1.0)[a]	Distance (Å)
430 GLN (430)	**NE2 ->**	**431 ASP (431)**	**OD1**	**Val= 0.602**	**DA= 2.99**
431 ASP (431)	**N ->**	**427 ARG (427)**	**O**	**Val= 0.538**	**DA= 2.80**
465 LYS (465)	**N ->**	**431 ASP (431)**	**O**	**Val= 0.520**	**DA= 3.14**
431 ASN (431)	N ->	427 ARG (427)	O	Val= 0.538	DA= 2.80
431 ASN (431)	ND2 ->	430 GLN (430)	OE1	Val= 0.610	DA= 3.02
431 ASN (431)	ND2 ->	427 ARG (427)	O	Val= 0.416	DA= 2.85
465 LYS (465)	N ->	431 ASN (431)	O	Val= 0.520	DA= 3.14

Area 2: The stability of the autofluorescence signature for *E. coli* was analyzed based on exposure to Carvacrol, a phenolic compound present in oregano and thyme plant essential oils. *E coli* strain C600 (ATCC 47024) (a gift of Moses Vijayakumar, Oklahoma State University), and E. coli O157:H7 (two different strains) were frozen at -80°C in a Trypticase Soy Broth (TSB) containing a final concentration of 15% glycerol. For use in experiments, a 100 µl sample of thawed stock was inoculated into 100 ml TSB and incubated at 37 °C overnight in a shaker bath at 120 rpm.

A monochromatic-based spectrofluorimeter (Photon Technology, Princeton, NJ, USA) was used for fluorescence. This instrument uses a xenon arc lamp to illuminate a one-half meter monochromator. The output of the monochromator is focused on a sample chamber wherein a sample cuvette is placed. Emission from the sample cuvette was collected at an angle of 90 degrees to the excitation after passing through an emission monochromator. Collection of the data was performed using photon-counting and a Hammamatsu R920 photomultiplier tube. Photon counts were stored on magnetic media and later analyzed and plotted using S-Plus (Insightful, Seattle, WA, USA) and Prism. A digital filter was applied to the raw data to remove photon scatter less than 25 nm of the absolute value of the excitation wavelength less the emission wavelength. An additional digital filter was applied to the data to remove the emissions from doubling of the primary excitation wavelength.

For culture preparation, a 3 ml of the overnight culture was mixed and removed from the middle of the flask and centrifuged at 2000 X g for 5 min. The resulting supernatant was removed and the pellet was resuspended in 150 mM saline. After a second centrifugation at 2000 X g, the pellet was resuspended in 3.0 ml of either control (150 mM NaCl, 2% EtOH) or treatment (0.01, 0.1, 1.0 mM carvacrol, 15.0 mM NaCl, 2% EtOH) in a polystyrene fluorimeter cuvette. Both control and treatment samples were maintained at room temperature. Optical density (absorbance at 660 nm) measurements (Ocean Optics S2000, Ocean City, MD, USA) were performed on each treatment before and after measuring autofluorescence to insure equivalent numbers of bacteria in control and treatment samples. After 15 min of incubation at room temperature in either control or carvacrol treatment, the cuvette containing the bacterial sample was placed in the sample chamber of a spectrofluorimeter (Photon Technology, Princeton, NJ, USA) and fluorescence was measured using excitation wavelengths of 300-700 nm and 400-700 nm emission. Fluorescence data was acquired by a computer, stored on magnetic media, and processed as three-dimensional plots using S-Plus (Insightful, Seattle, USA). All fluorescence scans were referenced to a factory fluorescent-calibration standard (Photon Technology Inc, Princeton, NJ, USA).

Figure 7 represents autofluorescence data from *E. coli* that was treated without carvacrol (control) or various concentrations of carvacrol. One axis of Figure 7 represents the excitation wavelength, which varied from 300 nm to 700 run, and the second axis represents the emission wavelength, which varied from 400 nm to 700 nm. The vertical axis of Figure 7 represents the fluorescence from the bacterial sample and is in volts, representing the number of photons emitted. Figure 7 is divided into four panels representing the control autofluorescence and the autofluorescence of *E. coli* exposed to increasing concentrations of carvacrol. Figure 7A, the control autofluorescence panel, shows complex peaks of autofluorescence uniquely characteristic of the C600 strain of *E. coli* and a characteristic trough near 550 nm emission for all excitation wavelengths. Figures 7B, 7C, and 7D autofluorescence data from E. coli treated with 0.01 mM carvacrol, shows little change in the overall signature based on the

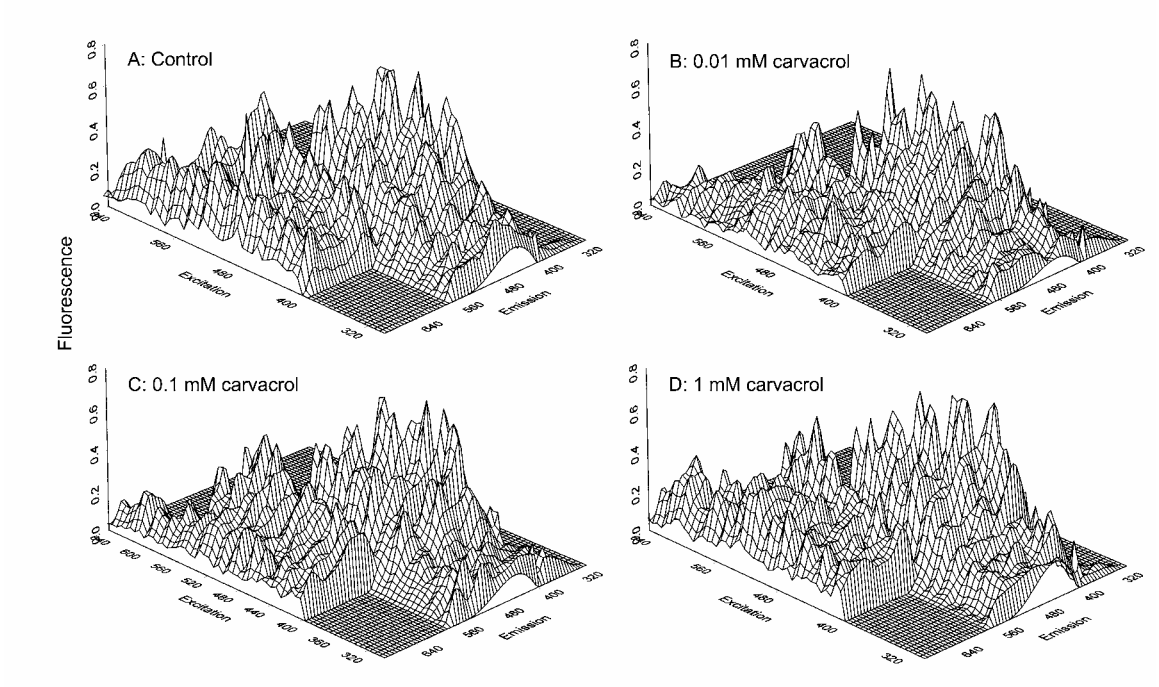

Figure 7. Three-dimensional representation of autofluorescence of E. coli exposed to the control (Fig. 7A) and 0.01 (Fig. 7B), 0.1 (Fig. 7C), and 1.0 mM (Fig. 7D) carvacrol. Excitation (axis leading away from observer and marked Excitation) ranged from 300 to 700 nm while emission (axis appearing flat) ranged from 400 to 700 nm.

We also presented the results as subtractions between control and treatments (difference spectra) in the panels of Figure 8. Figure 8A shows a control autofluorescence. Fig. 8B is the algebraic difference between the control autofluorescence spectrum and the 0.01 mM carvacrol spectrum (see Figure. 7B).

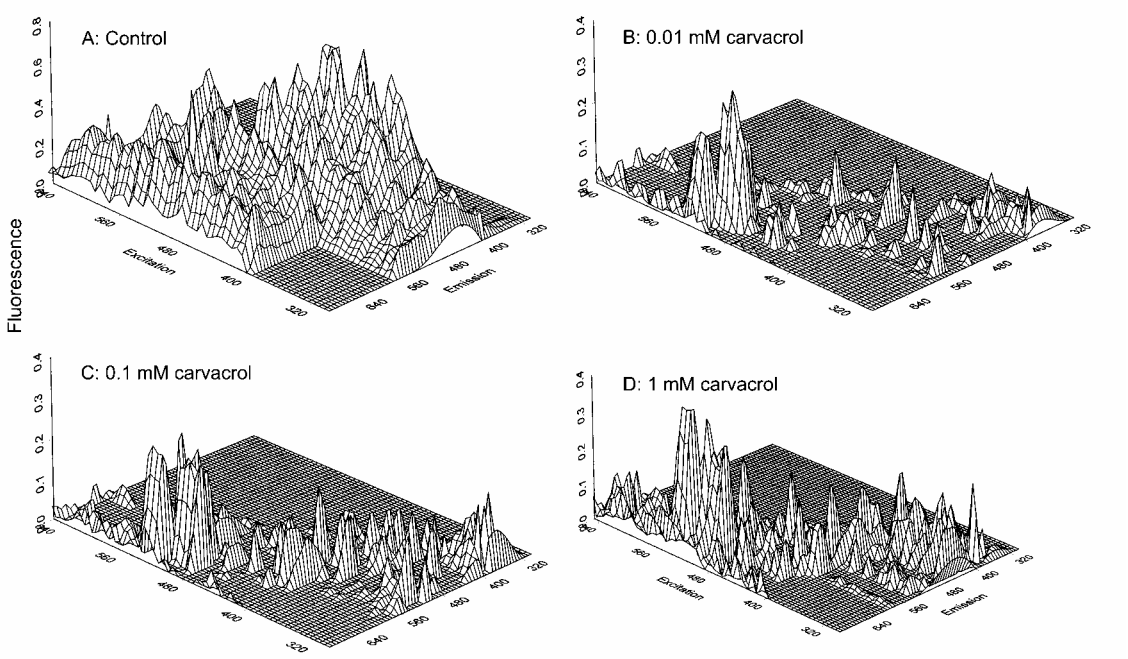

Figure 8. Three-dimensional representation of the autofluorescence of E. coli with control subtracted from each of the treatments (difference spectra): (Fig. 8A), control; (Figure 8B), 0.01 mM carvacrol less control; (Figure 8C), 0.1 mM carvacrol less control; and (Figure 8D), 1.0 mM carvacrol less control.

Although some changes did occur based on exposure to carvacol, overall there are peaks that remain stable, which is represented by flat or no peak formations in figures B, C, and D. Therefore, a stable autofluorescence signature for *E. coli* exist.

Further analysis of spectra data involved using the neural network system (13). Neural networks, an emerging machine learning approach, can perform highly complex mappings on noisy and nonlinear data, thereby inferring subtle relationships between sets of input and output parameters. They can in addition generalize from a limited quantity of training data to overall trends in functional relationships. Although several network architectures and training algorithms are available, the back-propagation type remains the most popular in bioinformatics applications (3). Feed-forward neural networks trained by back-propagation algorithm consist of several layers of simple processing elements called neurons, interconnections, and weights that are assigned to the interconnections. These rudimentary processors are interconnected in such a way that information relevant to the input-output mapping is stored implicitly in the weights. Each neuron contains the weighted sum of its inputs filtered by a sigmoid transfer function, endowing neural networks with the ability to generalize with an added degree of freedom not available in any statistical regression techniques. The input layer of neurons receives the external information such as the difference spectrum. The output layer transmits information to the outside world and this corresponds to the specific xenobiotics binded. Back-Propagation networks also incorporate one or more hidden layers of neurons which do not interact with the outside world, but assist in performing classification and nonlinear feature extraction tasks on information provided by the input and output layers. Neural network can be easily implemented in software, hardware or firmware, as appropriate.

The ability of real-time processing, noise rejection and continuous learning when more data become available make it a perfect tool for data analysis proposed herein. A nonpathogenic and two different strains of a pathogenic *E. coli* culture were analyzed using scanned data information. The different scans were analyzed and compared based on the number of data points having the same outputs (with a 5% threshold), which demonstrated a metric for comparison similar to regression analysis or sum square error analysis. Approximately 30% commonality exist between nonpathogenic and pathogenic *E. coli* (Table I). Approximately 60% commonality exist between the two strains of pathogenic *E. coli* (Table I). Based on the results, a distinction between nonpathogenic and pathogenic bacteria can be made. In addition, there is sufficient difference between the different stains of pathogens.

Table I

Percentage of commonality between scan 1, scan 2 & scan 3 of DISK 765

Scan 1 and scan 2	Scan 1 and scan 3	Scan 2 and scan 3	Scan 1,scan 2 & scan 3
30.22 %	30.15 %	60.62 %	30.15 %

Conclusion:

1.) Computer graphics modeling programs were used to determine the most ideal residues for mutation in order to increase stability of the CYP 3A4 protein. Future CYP proteins can be modified using the developed method.
2.) An emission/excitation spectrometer was build to generate 3-D plots from different species of bacteria. Nonpathogeneic and pathogenic strains of bacteria were tested. The results support the potential of autofluourescence signatures serving as method of identifying and distinguishing between different types of bacteria
3.) The preliminary data generated has enabled the submission of a larger grant to the National Science Foundation (Sensors and Sensor Networks), Program Solicitation NSF 03-512. Pending.

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1.) Brian C. Decocq, James T. Blankemeyer, Kristen R. Workman, and Mendel Friedman. Effect of Carvacrol on Autofluorescence, Membrane Potential, and ATP Flux of *Escherichia coli* C600, J. Appl. Microbiol., submitted 2004.
2.) Decocq, B. and G. John. Department of Microbiology & Molecular Genetics, Seminar Series, 2003.

E. STUDENT SUPPORT

1.) Brian Decocq- Master student, Department of Microbiology & Molecular Genetics- Autoflourescence work.
2.) Sanga Venkatraman- Master Student, School of Electrical and Computer Engineering
3.) Sumit Punj-Ph.D. student, Department of Microbiology & Molecular Genetics

Facilitating the Tenkiller Utilities Authority Public Water Decision Project

Basic Information

Title:	Facilitating the Tenkiller Utilities Authority Public Water Decision Project
Project Number:	2003OK28B
Start Date:	3/1/2003
End Date:	2/28/2004
Funding Source:	104B
Congressional District:	1st and 2nd
Research Category:	None
Focus Category:	Water Quality, Water Quantity, Water Supply
Descriptors:	None
Principal Investigators:	Mac McCrory, Weldon Schieffer

Publication

1. Decocq, Brian C., James T. Blankemeyer, Kristen R. Workman, and Mendel Friedman, 2004 (submitted) , Effect of Carvacrol on Autofluorescence, Membrane Potential, and ATP Flux of Escherichia coli C600, J. Appl. Microbiol.
2. Decocq, B. and G. John. 2003. Department of Microbiology & Molecular Genetics, Oklahoma State University, Seminar Series.

Interim Annual Report

Facilitating the Tenkiller Utilities Authority Public Water Decision Project
Oklahoma Water Resources Research Institute (2003OK19B)
July 29, 2004

The Institute of Issue Management and Alternative Dispute Resolution (IIMADR) was created within the OSU Seretean Wellness Center by Oklahoma Statutes (70 O.S. §3430) enacted by the Oklahoma Legislature in the spring of 2002. According to this statute, IIMADR provides:

> Issue management and alternative dispute resolution services and activities for agriculture, rural living, agribusiness, environmental, natural resources, and rural business or industry issues. The Institute is authorized to deliver issue management and alternative dispute resolution services and related activities to individuals, organizations, local, state, and federal government agencies, Native American tribes, and others that have an interest in or need for such services and activities.

The scope of services that IIMADR may provide to these entities include: collaborative discussion, deliberation, issue management, conflict prevention, dispute resolution, communication, training, and decision making. IIMADR was also charged with operating the Oklahoma Agricultural Mediation Program (OAMP) and the program is housed within the Institute.

The U.S. Army Corps of Engineers assessment report of 2001 found that the three-county Lake Tenkiller region of northeastern Oklahoma lacked adequate water storage and distribution capacity to serve the current population of the region. The region's population is projected to increase rapidly due to the desirability of the area as a retirement and recreation location.

At the same time that the region has exceeded service capacity, Lake Tenkiller's water quality has been affected by concentrated animal production, increased wastewater discharges, and the demands of industrialization within the watershed. The region was traditionally the poorest area in the state of Oklahoma and any economic development no matter how adverse was welcomed by the local citizens. By the 1980s the degradation of water resources had become apparent. In 1992, the Oklahoma Legislature enacted a resolution establishing a commission to investigate methods for solving the dilemma of natural resources and economic development in eastern Oklahoma.

Two municipal water systems and over thirty rural water districts serving nearly 100,000 people in this region participated in establishing the Tenkiller Utilities Authority (TUA) as a trust in 1995. The concept of the TUA was to centralize water supply and treatment for the region's water systems. The purpose of the TUA project is:

1. serving to prevent political units from competing for water storage, water rights, and the struggles for independent funding and compliance;
2. creating collaboration and partnering for the benefit of the entire the region;
3. preventing costly litigation and harsh competition among resource-strapped government offices; and

4. generating more productive, direct methods of addressing both social and economic issues.

For nearly a decade the TUA floundered as parties attempted to work together and vied for state and federal government grants to fund the project. Two years ago, TUA contacted IIMADR for the purpose of facilitating a dialog among the various participants. The mission of IIMADR, mandated in its enabling legislation enacted by the Oklahoma Legislature, was well-suited for providing needed direction to the TUA project.

Project Objectives

The project proposal outlined a statement of critical regional or state water problem; a statement of results or benefits and the nature, scope and objectives of the project; established a timetable for the project; outlined methods, procedures, and facilities of the project; and commented on related research and training potental.

Among the objectives outlined in the project proposal, IIMADR was responsible for:

1. assembling various data related to the project, based on geographical considerations, political boundaries, population densities, natural resource availability, census figures and other published projections, and developing computer data bases for use by the stakeholders;
2. assembling and neutrally disseminating contact data on and for stakeholders choosing to join in the IIMADR efforts;
3. planning, organizing, marketing, publicizing, and convening preliminary stakeholder meetings regarding TUA's water project, its long-term planning and cost recovery, and all facets of construction;

4. surveying and documenting consumer preferences and other stakeholder dynamics involved in the project; and,
5. neutrally engaging stakeholders in the direction and scope they choose to take the project.

Research Methodology

The purpose of this research was to evaluate the application of the issue management processes to avert a regional water dispute involving the Lake Tenkiller Utilities Authority, a public water supply, treatment and distribution system in northeastern Oklahoma. The study is within the mandate of the orginal grant application (2003OK19B) to "survey and document consumer preferences and other stakeholder dynamics within TUA's project."

The research methodology utilized for the evaluation was the case study method. A case study is a qualitative research method that is useful for the study of an organization, program, or project and understanding the effectiveness, interaction or dynamics of the organization, program or project.

The research question to be addressed is: How did the utilization of the issue management process work in averting a water supply dispute in the Lake Tenkiller region?

The research will provide funding agencies, facilitators, and scholars of facilitation and conflict resolution efforts a case study of the successes, weaknesses, opportunities, and threats of a facilitation of a dispute over natural resources in a rapidly-developing rural area. Research findings may also be used by the participants of the study and their agencies in future facilitation processes.

There were two groups of stakeholders in the Tenkiller Utilities Authority asked to participate in the study. One group consisted of members of the board of directors of the Tenkiller Utilities Authority who were directly involved in the facilitation process. A second group consisted of policy-makers who serve on the governing bodies of the municipalities and rural water districts within the region. These agencies selected representatives that served on the Authority's board of directors.

Interviews of Group I were conducted in November and December 2003 and in January 2004. The sampling method employed in this study was critical case sampling. Critical case sampling is a qualitative design method that permits logical generalization and maximum application of the sample case to other cases. Group I consists of all 30 members of the board of directors of the Tenkiller Utilities Authority. The Tenkiller Utilities Authority board members yielded the most information on issue management and had the greatest impact on the development of knowledge in the field of issue management.

Group I participants were interviewed in a face-to-face interview with open-ended questions. The interview was the standardized open-ended interview approach. The interview insturment consisted of questions that were written in advance of the interview. The exact interview instrument used in the evaluation was available for inspection by those who will use the findings of the study. All of the interviews were conducted in the individual offices or homes of the study participants.

The interviews were taped. After the interviewer returned to the IIMADR office the tapes were transcribed along with field notes from the interviewer about the interview.

The interview transcipts were analyzed by assigning codes to contiguous units of the transcipt text. The coding marked off fixed units of the text for later retrieval and indexing. Analytical statements were developed out of the coded transcipts.

Meeting Project Objectives

IIMADR according to the following narrative met the objectives of the IIMADR Tenkiller project proposals:

Objective One: Project Data Base

- Assembling various data related to the project, based on geographical considerations, political boundaries, population densities, natural resource availability, census figures and other published projections, and developing computer data bases for use by the stakeholders.

IIMADR met this objective by providing information to stakeholders at facilitation meetings, bringing together engineers and other natural resources and/or consultants to provide consultation to the TUA Board of Directors, and coordinating the flow of information to federal and state policymakers. IIMADR was credible as a neutral party. The Institute was credible because it was associated with OSU. Its neutrality put those involved at ease. It discouraged participants from having "an attitude." Some particpants hoped involvement with IIMADR would bring in grant money to their project.

A board member observed how IIMADR's data base was appreciated by the stakeholders: "[The IIMADR program manager] being an errand boy to the various agencies which none of us had the time or effort or initial contact to make these contacts with Oklahoma water systems and congressional representatives."

Objective Two: Stakeholder Contact Data

- Assembling and neutrally disseminating contact data on and for stateholders choosing to join in the IIMADR efforts.

IIMADR met the second project proposal objective by developing and maintaining a computerized data base on the stakeholders of the TUA project for use by all stakeholders.

Board members interviewed in the project evaluation used pharses and terms such as “credibility”, “disinterested party”, “expertise”, and “made them feel comfortable”, in describing how they felt about turning over information about themselves to IIMADR. IIMADR’s “disinterested third-party” status enabled “getting information out of people” and facilitated the “communications process”.

Objective Three: Stakeholders’ Meetings

- Planning, organizing, marketing, publicizing, and convening preliminary stateholder meetings regarding TUA’s water project, its long-term planning and cost recovery, and all facets of construction.

IIMADR met the third project objective by conducting two facilitation meetings in Tahlequah, Oklahoma in 2003. IIMADR, especially with the help of IIMADR’s program manager, got people talking, brought them closer together, and gained higher visability for the project. The meetings were attended by all of the stakeholders interested in TUA and the IIMADR facilitation process. Participants in the facilitation process believed that the preliminary stakeholder meetings helped focus the board members and was an

impetus for coalescing the members who represented the participating water systems for work on implementing the TUA concept.

A participant at one of the meetings, a representative of an interested federal agency, stated “you [IIMADR] did some of the due diligence in terms of trying to find out where things are, where all the different parties saw it, and where you needed to go...I think things have a better chance of happening now than they did a year ago.”

Another participant commented:

> The facilitation at least got the project moving...[it] got us looking at the project from a regional standpoint as opposed to what Cherokee Nation might think instead of just more of an idea of what could we do to benefit the entire area from utilization of Tenkiller water.

Objective Four: Consumer Preferences and Stakeholder Dynamics

- Surveying and documenting consumer preferences and other stakeholder dynamics involved in the project.

IIMADR accomplished Objective Four of the project by conducting an evalution of the TUA facilitation project. The results of the evaluation were not always flattering for the Institute. The perception was IIMADR seeemed to come late to the Tenkiller process and some participants found that, at first, it was not clear why they were involved in the process. Some of the issues of the TUA facilitation process were not fully covered, other issues were missed due to time constraints, and IIMADR was not always easy to reach by TUA stakeholders.

IIMADR’s credibility as a neutral thrid-party made it easy for the Institue to gather data but, according to one informant, stakeholders “had a little problem understanding

why they were there and what it was they were hoping to accomplish." The overwhelming perception in the words of one board member was IIMADR's presence was to "have the funds for us."

The IIMADR headquarter's distance from and travel time to the Tenkiller area was another factor in consumer dissatisfaction with the Institute. A stakeholder admitted:

> It seems like they're pretty well overloaded...we've tried to access [the IIMADR program manager] a few times and he was out of pocket and that gets a little frustrating...with any type of situation like this they are 150 miles away and not right here in the community, per se, I think...a weakness [is] in the speed in which the information could get to the people...Ii don't think that any agency that is not involved from the word go could go out here and communicate totally to the people what they've done and what the project is all about and being able to get back their feelings in a, should I say, systematic manner which could be transmitted into a working project.

Objective Five: Engaging the Stakeholders

- Neutrally engaging stakeholders in the direction and scope they choose to take the project.

IIMADR met the objective of engaging the stakeholders and directing them toward a goal or resolution for the project. Respondents in interviews believed that IIMADR brought people together to exchange information, brainstorm, find commom ground, and move a once stalled project forward. TUA members appreiated the neutrality that IIMADR contributed to resoluting impasses. He observed "basically you [IIMADR] come in as a third party and try to brainstorm solutions and manage them, I guess go through a process and break a logjam and bring something in to a point where it can be resolved."

The IIMADR involvement pushed the stakeholders to choose a direction for the program that many of the stakeholders believed they had the training to accomplish.

The engagement helped to develop direction for stakeholders as one board member describes:

> We had a bunch of people at that meeting who were workers in the rural water district...As a result they were just ordinary people and we were just ordinary people and we really did lack what I consider the ability to somehow mount an effort by someone to make this go. We just met and couldn't ever get it together. Jim Wilson came into that meeting, who is the state representative here, and that was one of the things he suggested that we somehow get off the pot and get moving and this had been going on for years and we just didn't seem to get it together and it was kinda discouraging to me that we really needed some help from someone who could give us help.

IIMADR enabled the stakeholders to develop a vision for the TUA project, in the words of one board member, "by assembling people and getting information from people that would assist in better understanding what the project is about, how its going to be implemented, and how it could be carried out."

Another stakeholder attributed a large role to IIMADR in providing guidance, stating, IIMADR was "probably a guiding light here…someone that we [could] come to and request assistance and [it was] there to give it to us."

The neutral engagement in assisting TUA participants in choosing a direction for the project was observed by one stakeholder as follows:

> Well it could be a lot of different things depending on the situation. [The IIMADR program manager] has on two occasions facilitated different agencies, different groups, employees as well as politicians being there…[T]hat facilitation part is what I appreciate.

The IIMADR facilitation process helped board members to see a workable program for the TUA project. Still another board member commented:

> In my opinion, [IIMADR was] trying to be a catalyst to progress, bringing information out to all the members . . . getting other people involved and getting people talking . . . . [Knowing] that there was money here and . . . that there was a research project

available with money to extend farther into the government, did give validity, that hey, this maybe is gonna go!

Timetable

The IIMADR activities specified in the timetable of overlapping activities were accomplished within the specific time periods.

Methods, Procedures, and Facilities

IIMADR utilized methods, procedures, and facilities for carrying out the project objectives in accordance with those methods, procedures, and facilities specified in the project proposal.

Related Research

The IIMADR project in conjunction with the Tenkiller Utilities Authority and the evaluation of this project were designed to add to research on the utilization of alternative dispute resolution processes relative to public sector utility projects in Oklahoma. Journal articles, conference presentations, and other publications will be developed from the data generated by this case study.

Training Potential

The IIMADR project, “Facilitating the Tenkiller Utilities Authority Public Water Decision Project,” provided opportunities for graduate and undergraduate students to be involved in the design, management, and business topics associated with environmental, economic, and other public utilities issues impacting eastern Oklahoma.

The project provided contacts for additional research in issue management and alternative dispute resolution and in related interdisciplinary studies.

Final Considerations

The evaluation of the TUA facilitation process was a qualitative study involving TUA board members and other TUA stakeholders who had firsthand knowledge of IIMADR activities. The study involved in-depth interviews with open-ended questions written in advance of the interviews and with follow-up questions to clarify responses.

A further research opportunity exists to expand the data by administering a Likert-scale survey. This qualitative survey would be administered to an expanded sample consisting of water system policymakers who are potential wholesale consumers of TUA and a sample of retail customers of the water systems themselves. This proposed study would generate in-depth data on the preferences and dynamics of proposed users of the TUA project.

Information Transfer Program

Activities for the efficient transfer and retrieval of information are an important part of the Environmental Institute/OWRRI program mandate. The Institute maintains a web site on the Internet at URL http://environ.okstate.edu/ that provides information on the OWRRI and supported research. The site provides links to information on publications of the Institute, grant opportunities and deadlines and any upcoming events. A listing of technical reports and other publications generated by OWRRI and other Environmental Institute sponsored research is updated regularly and is accessible on the Institute web site. Abstracts of each publication are available.

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 RCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	2	0	0	0	2
Masters	3	0	0	0	3
Ph.D.	3	0	0	0	3
Post-Doc.	0	0	0	0	0
Total	8	0	0	0	8

Notable Awards and Achievements

Publications from Prior Projects