2005 ANNUAL REPORT

Submitted to:

Director
Environmental Services Department
Arcata, CA

Submitted by:

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INTRODUCTION

In October of 2001 Humboldt State University (HSU), College of Natural Resources and Sciences (CNRS), Humboldt

State University Foundation (HSUF), and city of Arcata (City) signed a 10-year Memorandum of Understanding (MOU) effective retroactively from July 2001. This MOU was related in part to the conduct and funding of the Wastewater Utilization (WWU) Program at HSU. The purpose of the MOU is "to provide for cooperation and enhanced communication between the City, University, and College for the conduct of student investigations related to the City's aquaculture facility, composting, drainage, streams, storm water, wastewater treatment system, and wetlands, hereafter referred to as Water/Wastewater and Natural Resources & Sciences (W/WW & NRS). Results from student investigations are expected to contribute technical information needed by the City to better understand and improve the wastewater treatment system, maintain stream and drainage water quality, operate the aquaculture facility to minimize wastewater treatment costs, promote the beneficial reuses of wastewater, and contribute to the education of students". In return for investigative and support services performed by HSU employees, the City provides salary support and funding to conduct studies on wastewater treatment and aquaculture, wetlands, and stream and drainage water quality matters. Under this agreement, HSUF contracts with the city of Arcata to perform the duties described in the 2001 MOU.

This is the fifth annual report presented to the City, under the terms of the 2001 MOU, concerning activities of the WWU Program at HSU. In the following, sections are presented (1) a list of principal participants in the WWU Program, (2) a summary of activities related to wastewater treatment/utilization and to urban stream and drainage-water quality that were conducted during 2005, and (3) an outline of projects planned for the calendar year 2006 are

discussed. These proposed projects are presented under "Proposed Activities for 2006".

PRINCIPAL PARTICIPANTS

During 2005, the following faculty/part-time/staff members participated in the HSU Wastewater Utilization Program:

Dr. Kristine Brenneman,	Department of Fisheries Biology
Dr. Margaret Lang,	Environmental Resources and Engineering Department
Dr. Carol Lasko,	Chemistry Department
Dr. Eric Loudenslager,	Fish Hatchery Manager
Ms. Linda Mowry Wise	Lecturer, Department of Fisheries Biology

Dr. Kristine Brenneman served as WWU Coordinator from January to December 2005. In accordance with the 2001 MOU, Dr. Brenneman was employed 0.75-time by the University and the City paid 0.25-time of her salary through the Arcata Wastewater Account funds provided by the City to HSU and

distributed through the HSU Foundation. In 2005, duties of the coordinator were to (1) coordinate the HSU WWU Program students, (2) teach classes related to the treatment and use of wastewater and water quality, (3) conduct studies and direct undergraduate and graduate student investigations related to the City's wastewater treatment system and other water quality matters, and (4) seek extra mural support for such studies. This is in accordance with the MOU between the City and the University and in accordance with the 2001 agreement between the City and the HSU Foundation.

Dr. Margaret Lang provided assistance by reviewing and editing graduate student research theses in areas related to wastewater treatment and stream drainage systems. Dr. Lang currently is on Mr. Cory Jones' graduate committee. Dr. Lang has continued to review applicant files for the Wastewater Utilization Graduate Program. Dr. Lang attends monthly City meetings providing managerial information and technical support on local stream and watershed issues. Some meetings have conflicted with her teaching schedule and were unable to attend.

Dr. Eric Loudenslager supervised the rearing of rainbow trout for use in the toxicity tests for the City. His salary and costs of operating the HSU Fish Hatchery were not compensated by City funding. However, salmonid eggs for use in bioassay assessments were purchased through City funding. Rainbow trout eggs provided genetic diversity to HSU hatchery stock. Eggs were also used in the fish physiology course taught by Dr. Helen Mulligan.

Dr. Carol Lasko provided assistance by reviewing Wastewater Utilization graduate applicant files. Dr. Lasko

directs her analytical chemistry course to include metal and nutrient concentration analysis at the wastewater plant and aquaculture facilities.

Ms. Linda Mowry Wise taught Limnology Laboratory (FISH 320L) to cover for my overload. My new position, in addition to Fisheries Biology Professor, is Department Chair of Native American Studies.

ACTIVITIES DURING 2005

Administrative/Operations in 2005

1. In March of 2005, Dr. Kristine Brenneman prepared and submitted a budget for fiscal year 2005-2006 to the City. **APPENDIX I**.

2. Due to receiving late student reports, Dr. Brenneman prepared and submitted in June, 2005, an annual report to the City for the calendar year 2004 in accordance to the stipulations within the 2001 Memorandum of Understanding.

3. Dr. Brenneman continued administering the Master's of Science in Natural Resources and Science, Wastewater Utilization option, within the HSU College of Natural Resources and Sciences. During 2005 the following graduate students were enrolled in WWU graduate program: 1) Nick Simpson, 2) Jennifer Cole, 3) Corey Jones, 4) Sarah Muskopf, 5) Holly Tretten, 6) Kasey Sirkin, 7) Patrick Wiley, 8) Karen Bromley and 9) Elisa Soltren.

4. Dr. Brenneman conducted undergraduate studies pertaining to wastewater treatment and urban stream water quality issues as well as wastewater aquaculture. During 2005 the following undergraduate students were: 1) Alfonso Campa, 2) Jared Kurtz, 3) Aryan Dilts, 4) Mike Eakin, and 5) Jason Soto.

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5. At the Arcata wastewater-seawater aquaculture ponds some projects were completed during 2005 work year. An outline of the activities are listed below under line item #6 a-l. **Appendix II**

6. Student assistants at AWWAP performed the following tasks as part of the wastewater aquaculture component of the agreement:

   a. Maintained/upgraded facilities at the AWWAP. This included replacing equipment such as pumps and aerators. Improvements included painted the hatchery exterior, otter deterrents by repairing electric fence, repaired fencing around summer ponds, and painted inside hatchery.

   b. continued to remove old materials and pampas grass around the hatchery and fish barn, and cleaned and repaired fish tanks.

c. Increased salinity of yearling ponds once the salt water pump was replaced to eliminate *Azolla* (mosquito fern).

d. In March 2005, 2,200 steelhead fry and 1,200 coastal cutthroat fry were obtained from HSU Fish Hatchery.

e. Coastal cutthroat (544) and steelhead trout (446) fingerlings were transferred to SP1 and SP2 in September and continue to be reared in outdoor ponds using a constant flow of treated wastewater/seawater. Stock is too young to spawn in January 2006 but will ripen next spawning period.

f. Received approximately 1,000 rainbow trout fry from the HSU Hatchery Facility to rear in the fish barn for bioassay.

g. One white sturgeon (*Ascerpensor transmontanus*) acquired from UC Davis died due to lack of care of the outside tank by Simpson. Attempts by Jason Ogawa to acquire more sturgeon from Dr. Chech from UC will not happen because their

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contract with the tribe expired. Dr. Chech is also retiring.

h. Jolly Giant Creek fish weir was in operation. No adults had been trapped. Vandalism occurred three times which were repaired immediately. One incident was comprised of an eight square inch hole cut in the bottom of the trap.

i. Tours were given to the following schools, interested people, and organizations: HSU Aquaculture FISH 370 (Fisheries Biology Department, Dr. Hendrickson); Mr. and Mrs. Robert Mobley; Dr. Carol Lasko, HSU Chemistry Department; HSU Fish Disease FISH 525 class (Dr. Hendrickson); Mike Wallace (Department of Fish and Game). For complete list of visitors and dates see **Appendix III.**

j. From May of 2004 to February of 2005, 15 stickleback and mosquito fish were held in dechlorinated effluent without feeding and survived the entire period.

k. Two IACUC inspections were held, March 24, 2005, and October 11, 2005, with no violations found.

The first inspection was attended by almost every board member but now only two inspectors show which I believe speaks volumes to the facility following proper protocol for the care and rearing of salmonids in a hatchery setting despite unsubstantiated statements by M. Shoemaker.

l. Stocking of coastal cutthroat into Janes Creek at the confluent and Jolly Giant Creek above the trap was postponed due to low numbers of fry. Likewise, due to seriously low numbers of steelhead fry left in AWWAP, prevented any stocking activities into Janes, Jolly Giant and Campbell creeks.

8. City personnel performed the following repairs and upgrades to the aquaculture facility:

a. New salt water pump.

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b. New exhaust ventilation system for generator was installed for safety reasons as required by OSHA.

9. Aquaculture and Klopp Lake water quality monitoring program was started in fall for 2005. Tests included pH, dissolved oxygen, nitrite, ammonia, temperature, conductivity, and salinity. Due to personnel mismanagement and misconduct water quality tests were not performed on a regular basis. **Appendix IV.**

10. Dr. Eric Loudenslager continued to rear rainbow trout from eggs to fingerlings for use in wetland effluent toxicity testing.

11. Dr. Brenneman prepared a Wastewater Utilization Program Inventory. List is attached as **Appendix V**.

Projects Conducted in 2005

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1. Patrick Wiley (graduate student assistant), under the direction of Dr. Brenneman, carried out **nitrogen analysis** in the Arcata wastewater treatment system. This is an ongoing study to investigate the nitrogen budget in the wastewater treatment system. Samples were collected from 16 sites and tested for ammonia and nitrate on a weekly basis. Additionally, temperature and pH readings were taken on-site and recorded. The system is effectively reducing nitrate and ammonia concentrations. Results are attached as **Appendix VI**.

2. Patrick Wiley (graduate student assistant), under the direction of Dr. Brenneman, carried out **hydrogen sulfide analysis** in the Arcata wastewater treatment system. The concentration of hydrogen sulfide was within the normal range but higher in the treatment marshes as expected. Even though removal was due to chlorination there was a general trend of reduction throughout the treatment train. **Appendix VI**.

3. Jennifer Cole, Elisa Soltren and Holly Tretten (graduate student assistants), under the supervision of Dr. Brenneman, monitored the water quality of Jolly Giant, Janes, Campbell, and Groztman creeks. Dissolved oxygen (DO), temperature, and pH were monitored on a weekly basis. Biochemical oxygen demand (BOD) and

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fecal coliform tests were measured monthly. **Appendix VII/VIII**.

4. Alfonso Campa (undergraduate student assistant), under the supervision of Dr. Brenneman, continued the acute toxicity bioassay of wetland effluent using rainbow trout, *Daphnia*, and mosquito fish (*Gambusia*). There is a water quality problem. He was in a serious automobile accident and the position was given to Nick Simpson who did not perform the tests for five months. Report is for part of 2005 attached as **Appendix IX.**

5. Nick Simpson (graduate student assistant) directed by Dr. Brenneman, sampled mosquito populations in the polishing marshes, treatment marshes and Aldergrove Marsh. Habitat, food preference, and mosquito control by three-spine stickleback and mosquito fish has been completed. Report is attached as **Appendix X**.

6. Alfonso Campa (undergraduate student assistant), directed by Dr. Brenneman, has continued to raise *Daphnia* in illuminated fish hatchery water to provide a ready supply for bioassessment use. After Alfonso's

accident, Nick Simpson has taken over this project. Populations are occasionally replaced through Nasco (Modesto, CA)

7. Aryan Dilts and Jason Soto (undergraduate student assistants) directed by Dr.Brenneman and Dr. Lasko, assessed SP and YP ponds for the following metals:

   Lead, copper, zinc, and cadmium using Hach methods. The students created a poster and is displayed on the second floor bulletin board of the Wildlife Fisheries Building. Report is on file in 2004 Annual Report.

Courses Taught Related to Wastewater Utilization and Water Quality in 2005.

1. Limnology (FISH 320). Dr. Kristine Brenneman, Spring 2005.

2. Wastewater Ecosystems Analysis and Reuse (FISH 525) was not taught because of sabbatical conflicts. Dr. Kristine Brenneman, Spring 2005

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3. Limnology (FISH 320). Dr. Kristine Brenneman, Fall 2005.

4. Limnology Laboratory (FISH 320L). Linda Mowry Wise, a former graduate of the WWU graduate Program, Fall 2005.

5. Water Quality Laboratory (ENGR 350). Dr. Margaret Lang, Spring and Fall 2005.

Grants for 2005

1. Department of Energy. $22,000. – Funded (Air Sampler) **Appendix XI.**

2. Faculty Diversity Grant. Submitted for $1,000 – $500 Funded. **Appendix XII.**

3. Friends of the Arcata Marsh. Tretten and Brenneman, $745 – Funded (Metals in fish tissue)

4. Friends of the Arcata Marsh. Jones and Brenneman, $175 – Funded (*Lemna* uptake of nitrogen in the presence of antibiotics)

Workshops/Professional Meetings Attended in 2005.

- Dr. Brenneman and the following students attended the Environmental Law Conference, Eugene, Oregon, March, 2005: Cory Jones, Patrick Wiley, and Nick Simpson. This meeting provided an opportunity for students to network with professionals in their field and access information important to their education. Travel, registration, lodging, and meals were funded through the city of Arcata WWU grant.

- During the ELaw Conference, 2005, the three WWU graduate students named above and Dr. Brenneman took a tour of the wastewater treatment facility in Eugene, OR. The plant operators gave us a TWO HOUR tour. Dr. Brenneman wrote a letter of appreciation. **Appendix XIII.**

- E. Soltren and K. J. Brenneman, Zinc removal from oil-water separator effluent using chitosan. Presented at the Society for the Advancement of Chicanos and Native Americans in Science (SACNAS) national meeting in Denver, Colorado, October 1, 2005. **Appendix XIV.**

- N. Simpson and K.J. Brenneman. 2005. Mosquito predation by two fish species in the Arcata polishing marshes and Aldergrove Marsh. Presentation at the biannual Upsets meeting - City WWU invitation meeting. CD copy attached as **Appendix XV.**

- Attended the HSU's President's Native American Advisory council meeting as the Chair of Native American Studies Department on October 3, 2005. The Yurok and Karuk Tribes in Weitchpec Yurok Tribal Office hosted the meeting.

- Dr. Brenneman was part of the planning committee for the 20th Annual California Indian Conference in October 2005.

Master's Theses Completed in 2005

- Kasey Sirkin. Thesis draft titled " Evaluation of the correlation between aquatic macroinvertebrate populations and fecal coliform concentrations to assess water quality of four coastal streams in northern California". See 2004 Annual Report.

Awards/ Publications/ Reviews in 2005

- HERO Award winner: North Coast Pollution Prevention Committee awarded Drs. Brenneman and Lasko the Pollution Prevention HERO (Humboldt Environmentally Responsible Organization) Award which honor outstanding pollution prevention efforts. Press release copy attached as **Appendix XVI.**

- Reviewed a manuscript for the Canadian Journal of Microbiology concerning a proposed article entitled "An optimized enumeration method for Sorbitol-Fermenting Bifidobacteria in environmental samples". I believe I was invited to review this article based on WWU graduate

Sommers' study for the City concerning Bifidobacteria. The article was accepted.

- Participated as an abstract reviewer for the Student Poster and Oral Presentations for SACNAS (Society for the Advancement of Chicanos and Native Americans in Science).

- IACUAC approved form for Nick Simpson, graduate student under the direction of Dr. Brenneman, to carry out his research on mosquito predation by mosquito fish and stickleback. **Appendix XVII**

Community Service for 2005

1. Nick Simpson, graduate student under the direction of Dr. Brenneman, volunteered to work on a project with Hydesville Elementary School and Mr. Sal Steinberg, Friends of the Van Duzen River. He helped students with establishing water quality using rapid bioassessment monitoring techniques. **Appendix XVIII.**

2. Nick Simpson, graduate student under the direction of Dr. Brenneman, was asked by Mr. Rizza, Hydesville Elementary School and Mr. Sal Steinberg, Friends of the Van Duzen River, to teach and direct the students to perform water quality on Wolverton Creek, CA. **Appendix XIX.**

2. Provided steelhead fry to Mike Wallace, CA Department of Fish and Game, through American Fisheries Society, for REEF. REEF is the Redwood Environment Educational Fair that invites all school to participate in educational activities. It is so popular that schools participate by a lottery.

PROPOSED ACTIVITIES FOR 2006

Services to be Provided to the City in 2006:

1. Continue presentation of seminars, displays, and tours of the Arcata Wastewater Treatment System by Drs. Brenneman and Lang.

2. Technical assistance will be provided by Drs. Brenneman, Lasko, and Lang in the physical, chemical and biological analysis of water samples.

3. Technical assistance will continue to be provided by Dr. Brenneman for establishing a freshwater toxicity testing capability to conduct freshwater toxicity tests that will be required in the future on polishing marsh effluents and non point-source discharges within the city of Arcata.

4. Continue to rear rainbow trout from eggs to fry stage by Dr. Loudenslager at the HSU Fish Hatchery, for use in toxicity tests to be conducted on the polishing marsh effluent at the bioassay facility, AWWTP.

5. Reestablish stocking to Janes Creek, Jolly Giant Creek, and Campbell Creek with steelhead trout and cutthroat trout fry.

6. Continue operation of the AWWAP facility by Dr. Brenneman and student assistants, raising coastal cutthroat and steelhead trout, *Gambusia*, three-spined stickleback, and sturgeon.

7. Continue progress reports in the format of student presentations or written documents. It is hoped that sometime during the Spring semester the city of Arcata Environmental Services, HSU Wastewater Utilization and Environmental Engineering programs will hold the return of the student presentation series at the Arcata Marsh Interpretive Center. This was an open forum for the public, HSU faculty, staff, and students, and the City to acquire information about projects conducted in and around the city of Arcata. A tentative date was canceled for May 25, 2005.

8. Continue monthly meetings with city of Arcata Environmental Services staff to discuss issues pertaining to wastewater treatment and urban stream water quality subject matters.

9. Continue requirement by each student working under the WWU Program to provide Standard Operating Procedures (SOP) for tests they perform on a routine basis. Standard Operating Procedures submitted will continue to be available to the city of Arcata and are located in the Wastewater Utilization Program Laboratory, Wildlife/Fisheries Building, Room 272, Humboldt State University. Jared Kurtz, (Shawnee) undergraduate student under the direction of Dr. Kristine Brenneman, has completed a standard operating procedure for his position of water quality collection and testing. **Appendix XX.**

10. Continue weekly monitoring of the wastewater treatment facility at 16 sites for hydrogen sulfide, ammonia, nitrate, pH, temperature and dissolved oxygen.

11. Sustain water quality testing throughout the wastewater aquaculture and Klopp. The water quality information will provide qualifying data to initiate upgrade procedures on the fish hatchery to maintain high State hatchery standards.

12. Continue educational programs by Dr. Brenneman to introduce middle school children to field science through water quality testing. The aquaculture facility will continue to be the permanent field site to introduce the principles of water quality testing through sampling the various fish ponds and analyzing the results. McKinleyville Middle School has adopted SP3 and will be conducting water quality tests and raising fish.

13. Carry on Total Kjeldahl Nitrogen testing throughout the wastewater treatment facility using the safer technique by Hach. It is not an EPA approved method.

14. Publish theses results with Andrew Jensen and Linda Mowry Wise.

Proposed Investigative Projects for 2006

1. The fish barn is completed with flow-through systems. Water quality testing and fish rearing will begin.

2. Raise mosquito fish and stickleback in controlled fish tanks to determine maximum production rate at the lowest cost. These fish will be used for bioassay and mosquito prey preference studies (Nick Simpson thesis). SEE APPENDIX XIII.

3. Raise white sturgeon in reclaimed wastewater. Sturgeon stock will be obtained from University of California, Davis. Growth and survival rates will be studied. Jason Ogawa, previous undergraduate student at AWWAP, has been in contact with the University of California, Davis to obtain sturgeon. He will be returning as a graduate student in Spring 2007.

4. Continue to examine the water quality of Klopp Lake. In the past, salinities have exceeded those levels necessary for survival of salmonids for stocking purposes. The water quality information will provide the City and wastewater aquaculture with data for management and stocking decisions.

5. Elisa Soltren, graduate student directed by Dr. Brenneman, will continue to monitor mosquito population densities in treatment marshes and wetland areas. High population densities can become a public nuisance and health hazard. Concern over the West Nile Virus has prompted the need for a more in-depth genera survey and population dynamics study.

6. Patrick Wiley, graduate student directed by Dr. Brenneman, will implement stream flow and discharge data collection stations at specified locations. This project was postponed until 2006 as part of the stream monitoring project.

7. Brian Exsberger (spelling?), graduate student directed by Dr. Casey Liu, is determining lead and zinc uptake in cattails grown in aqueous solution. See thesis proposal in 2002 Annual Report.

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8. Sarah Muskopf, graduate student directed by Dr. Brenneman, has completed the evaluation and data collection regarding the fate of total phosphorus in Taylor Creek Wetland in the Lake Tahoe Basin. Ms. Muskopf has submitted her first rough draft. See 2001 Annual Report for thesis proposal.

9. Karen Bromley, graduate student directed by Dr. Brenneman, proposes to examine biosolids from the clarifier, anaerobic digester, and drying beds for groups of organisms such as, protozoa, rotifers, and bacteria. Bacterial groups will include enterics

(*Salmonella* and *Shigella*) and enterococci (fecal streptococci). Thesis proposal has not been submitted. She will utilize the air sampler obtained from DOE.

10. Cory Jones, graduate student directed by Dr. Brenneman, will assess the effects of selected antibiotics on the ability of *Lemna* (duckweed) to uptake nitrogen. Thesis proposal is attached as Appendix **XXI**.

11. Elisa Soltren, graduate student directed by Dr. Brenneman, proposes to study the effects of septic tank systems along the Eel River for water quality impacts. This project is cosponsored (not funded) by the Humboldt Redwood State Parks. Thesis proposal is attached as **Appendix XXII**

12. Melissa Kreye, graduate student directed by Dr. Brenneman, proposes to study the effects of metal accumulation on fish gill epilethial and liver tissues. This was started by Holly Tretten but has now taken a leave of absence from the program for one year.

13. Nick Simpson, graduate student directed by Dr. Brenneman, has completed data collection and is currently analyzing the data and composing his thesis. The evaluation will aid the City to control mosquito outbreaks to safe levels. See 2004 Annual Report for thesis proposal.

14. Jennifer Cole Bidaurreta, graduate student directed by Dr. Brenneman, has completed her analysis and

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evaluation of antecedent precipitation indexing on Janes Creek and will be making the appropriate corrections on her first draft followed by resubmission of her second draft.

15. Patrick Wiley, graduate student directed by Dr. Brenneman, will assess the feasibility of biodiesel production from *Chlorella* isolated from AMWS polishing marsh ponds. This may aid the City to obtain an additional source of energy to operate the wastewater facility. Thesis proposal is attached as **Appendix XXIII.**

Community Service for 2005

1. Nick Simpson, graduate student under the direction of Dr. Brenneman, volunteered to work on a project with Hydesville Elementary School and Mr. Sal Steinberg, Friends of the Van Duzen River. He helped students with establishing water quality using rapid

bioassessment monitoring techniques. **Appendix XVII.**

2. Nick Simpson, graduate student under the direction of Dr. Brenneman, was asked by Mr. Rizza, Hydesville Elementary School and Mr. Sal Steinberg, Friends of the Van Duzen River, to teach and direct the students to perform water quality on Wolverton Creek, CA. **Appendix XVIII.**

2. Provided steelhead fry to Mike Wallace, CA Department of Fish and Game, through American Fisheries Society, for REEF. REEF is the Redwood Environment Educational Fair that invites all school to participate in educational activities. It is so popular that schools participate by a lottery.

2005 BIOASSAY ANNUAL REPORT

Prepared by:

Kristine J. Brenneman, Ph.D.
Wastewater Utilization Program Director
Humboldt State University

Student Technicians:

Alfonso Campa
Cory Jones
Nick Simpson

January 14, 2006

ABSTRACT

The purpose of this study was to bioassay the polishing marsh effluent from a secondary wastewater treatment plant. The monthly toxicity testing project was conducted at the Arcata Wastewater Treatment Plant and is the seventh year of toxicity testing. The design includes toxicity testing using early life stage rainbow trout (*Oncorhynchus mykiss*) in flow-through bioassays to provide future water quality discharge requirements. In addition to rainbow trout, mosquito fish (*Gambusia*) 96-hour acute continuous flow-through bioassays may be included but is not required. *Daphnia* 96-hour acute static renewal bioassays were conducted as an invertebrate species test. Results for 2005 suggested that, other than low dissolved oxygen content and high hydrogen sulfide concentrations during the mid-year months, polishing marsh effluent is of good quality as indicated by 96-hour acute bioassay survival of trout, *Daphnia*, and mosquito fish. Results were very much like that of 2004. Flow rates in bioassay test containers are being monitored closely for dissolved oxygen and hydrogen sulfide concentrations. In addition, chlorine testing has been conducted before and after each monthly bioassay. Feeding was implemented due to high mortality rates in the control. When new students took over, feeding was not performed even though they were instructed to do so. Results were similar to 2004 unfed controls.

2005 Bioassay Report - Wastewater Utilization Program

INTRODUCTION

Amendments to the 1972 Federal Water Pollution Control Act, more commonly known as the Clean Water Act, resulted in the new national policy direction "that the discharge of toxic pollutants in toxic amounts be prohibited". To implement the toxicity policy, the U.S. Environmental Protection Agency (EPA) is merging toxicity based discharge limits into the National Pollutant Discharge Elimination System (NPDES) permits. The Clean Water Act serves as the primary legal framework for the control of surface water pollution in the United States. Implementation of the Clean Water Act has relied primarily on developing regulatory criteria for individual chemicals. Because these criteria are based nearly exclusively on laboratory data, their relevancy under actual field conditions is unclear. Many effluents are variable and contain complex mixtures of toxicants. Thus, additive and synergistic affects are difficult to assess using individual chemical criteria. Likewise, temporal and seasonal variation in nature further complicates the assessment process. Therefore, NPDES permits have placed increased importance on biomonitoring.

Increased awareness of the value of effluent toxicity test data for toxic control prompted EPA to propose that toxicity data be used to assess and control the discharge of toxic substances to waters of the State through the NPDES permit program. The new EPA policy stated, "Biological testing of effluents is an important aspect of

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the water quality-based approach for controlling toxic pollutants. Acute and short term chronic effluent toxicity data, in conjunction with other data, can be used to establish control priorities, assess compliance with State water quality standards, and set permit limitations to

achieve those standards". Currently, EPA recommends that three species bioassay should be considered as one method of assessing compliance with narrative toxicity objectives. The preferred system of testing toxicity is a flow-through system but is currently rarely implemented. The system established by the Wastewater Utilization Program is a flow-through system.

The North Coast Regional Water Quality Control Board (Board) Basic Plan contains a narrative toxicity objective, which states, "All waters shall be maintained free of toxic substances in concentrations that produce detrimental physiological responses...in aquatic life". Under the Board's jurisdiction, Arcata Wastewater Treatment Plant (AWTP) receives, treats, and discharges domestic and industrial waste into the bay from approximately 14,000 people. Humboldt Bay, the receiving water, has been characterized as the cleanest and healthiest bay of California (Pequeqnat and Butler, 1982). The AWTP has a biological assay effluent requirement under the narrative toxicity objectives of the Board that is currently testing freshwater fish with 96-hour bioassays in undiluted effluent prior to discharge to the bay.

In addition to the City's bioassay, the student-run bioassay project is being conducted as part of an ongoing project with Humboldt State University's (HSU) Wastewater

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Utilization Program and the city of Arcata. The primary objective of this study is to conduct monthly 96-hour acute and short-term chronic bioassays using rainbow trout (*Oncorhyncus mykiss*) and Cladocera (*Daphnia* spp.) with a continuous flow-through and static systems, respectfully.

MATERIALS AND METHODS

All bioassays were run for 96 hours. Bioassay procedures are modeled after EPA (1993) and EPA (1994). Further explanation of the procedures can be found by consulting the Humboldt State University (HSU) Wastewater Utilization Program Bioassay Protocols.

Flow-through Vertebrate Bioassay Procedures

All fish bioassays were conducted using the flow-through bioassay system. The HSU Fish Hatchery receives rainbow trout (*Oncorhyncus mykiss*) eggs from Mt. Lassen Trout Farms, Red Bluff, California. Eggs are hatched quarterly and ten fish from each quarterly cohort are used for bioassays. Mosquito fish (*Gambusia*) were obtained from Humboldt County areas other than wastewater treatment systems and used for bioassay. Ten fish were used. Both mosquito fish and rainbow trout were simultaneously tested monthly when possible. Certain times of the year, mosquito fish were difficult to obtain and thus were not used. All fish that survived the toxicity testing were disposed of appropriately by requirements under the IACUC.

At one time, fish were transferred from HSU to the AWTP in plastic Ziploc® containers. The transport of fish

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have been modified so as to minimize stress. Fish are transported in an aerated transport-stocking tank. In the bioassay room, they are placed in aerated water (100 bubbles per minute) and left to acclimate before initiating the test. Aeration during the test was continuous.

Survival was monitored and recorded daily over 96 hours. Temperature, pH, hydrogen sulfide, chlorine, and dissolved oxygen were measured at initiation and termination of each test. Dissolved oxygen was measured with a calibrated Yellow Springs Instrument (YSI) dissolved oxygen meter, model 55. The pH was measured using a calibrated

Fisher Scientific Accumet pH meter 50. Hydrogen sulfide and chlorine were measured using Hach DR 890 colorimeter.

Static Renewal Invertebrate Bioassay Procedures

All invertebrate bioassays were conducted using the static renewal procedure. *Daphnia magna* were obtained from Nasco (Modesto, CA) when necessary. Otherwise, *Daphnia* were raised by A. Campa in order to obtain neonate age class. One to three day old *D. magna* neonates were used for static renewal experiments. Only one invertebrate species was tested during a monthly bioassay. Water samples were collected within six hours of test initiation and stored in HDPE Nalgene® one-liter containers at 4 °C. Sample water was warmed to 25 °C before loading with test species. One treatment with four replicates of 100% wetland effluent was used for each experiment along with HSU Fish Hatchery control water. Each sample vial

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contained 18 mls of wetland effluent and was renewed daily after a survival count. Cladoceran replicates contained ten organisms each. Experimentation without feeding regimes was conducted. Dissolved oxygen was never adjusted.

RESULTS

Wetland effluent temperature and dissolved oxygen changed seasonally (Table 1). Effluent temperatures ranged from 13.0 ^{o}C to 20.9 ^{o}C. Dissolved oxygen ranged from 1.25 mg/l to 8.3 mg/l. The pH ranged between 6.3 and 7.8 during this study. The pH of the control water ranged from 5.8 to 7.7, dissolved oxygen ranged from 2.5 mg/l to 10.25

Table 1. The 96-hour flow-through bioassay effluent data for 2005. The pH is an antilog mean. Feeding of organisms commenced in June 2005.

DATE	pH		**D.O. (mg/l)**		T (^{o}C)		**H_2S (mg/l)**	
	Start	End	Start	End	Start	End	Start	End
4/12/04	6.3	----	2.15	----	20.9	----	0.08	----
5/04/04	7.0	7.0	N/A	N/A	18.7	----	0.06	----
6/24/04	6.9	7.6	6.20	7.00	18.0	18.4	**0.60**	**0.80**
7/15/04	7.7	7.8	----	8.30	20.6	19.0	----	**7.20**
8/01/04	6.9	6.8	**1.98**	----	18.6	17.9	**3.6**	**----**
9/10/04	----	6.7	----	----	----	18.9	**----**	**0.80**
10/27/04	----	6.6	----	**2.80**	----	14.1	**----**	**0.40**
11/01/04	6.6	6.6	**2.80**	----	14.1	----	**0.40**	----
12/01/04	6.5	6.5	----	----	13.9	----	**----**	----
MEAN	**6.8**	**6.95**	**3.28**	**6.03**	**17.83**	**17.66**	**0.95**	**2.30**

mg/l = milligrams per liter
D.O. = dissolved oxygen
T (^{o}C) = temperature in degrees Celcius
H_2S = hydrogen sulfide

mg/l, temperature 14.0 ^{o}C to 21.2 ^{o}C. Low dissolved oxygen and high hydrogen sulfide concentrations have been the problem concerning survival of test organisms. Summer levels of hydrogen sulfide reached a deadly 7.2 mg/l. Effluent chlorine residual ranged from 0.0 to 0.14 mg/l whereas, the control ranged from 0.0 to 0.15 mg/l (Table 1 and

Table 2). The pH of both the effluent and control test waters were within normal limits for natural waters.

Table 2. The 96-hour flow-through bioassay control data for 2005. The pH is an antilog mean.

DATE	pH		D.O. (mg/l)		T (^oC)		CHLORINE (mg/l)	
	Start	End	Start	End	Start	End	Start	End
4/12/04	5.8		8.85		20.9	20.3	0.00	0.00
5/04/04	6.9	6.5	---	10.25	14.0	14.7	0.02	0.00
6/24/04	7.2	7.3	7.00	7.60	19.8	19.9	0.18	0.15
7/15/04	6.9	7.7	----	9.13	22.4	19.0	0.03	0.03
8/01/04	7.2	7.2	2.15	---	20.9	21.2	0.10	0.00
9/10/04	----	7.5	----	----	----	21.2	----	0.03
10/27/04	----	7.1	----	7.80	----	16.5	----	0.02
11/01/04	7.1	----	7.80	----	16.5	----	0.02	0.00
12/01/04	6.9	----	----	----	16.0	----	----	----
MEAN	**6.8**	**7.2**	**6.45**	**8.70**	**18.64**	**18.97**	**0.06**	**0.03**

mg/l = milligrams per liter
D.O. = dissolved oxygen
T (^oC) = temperature in degrees Celcius
H_2S = hydrogen sulfide

Fish Toxicity Testing

The Board requires that survival of test fish in 96 hour continuous flow-through bioassays in undiluted effluent samples shall equal or exceed 90% survival 67% of the time and 70% survival 100 percent of the time. During the study, rainbow trout survival in effluent test water ranged from to zero percent to 100% (Table 3). The times that results met EPA criteria was June and October through December, 2005.
All other months, survival rates were < 20%. Control survival rate was zero until October.

Table 3. Monthly 96-hour bioassay survival percentage rates on four species during 2005 on Arcata's treated wastewater effluent and control waters. Control water is municipal drinking water.

Date	**Mosquito Fish**		**Rainbow Trout**		*Daphnia*	
MONTH	EFF*	CON#	EFF	CON	EFF	CON
April	60	0	60	0	40	10
May	NA	NA	70	0	30	10
June	80	100	40	60	85	70
July	0	90	0	70	15	65
August	0	80	0	50	0	75
September	0	90	0	60	5	75
October	90	70	70	50	70	80
November	100	90	80	50	95	70
December	100	90	90	70	85	65

* EFF = effluent
# CON = control
NA = not performed

Mosquito fish (*Gambusia affinis*) bioassay procedures were performed simultaneously with rainbow trout bioassay tests. Mosquito fish survival rates in the effluent ranged from zero to 100% (Table 3). The control exhibited similar results with a range of survival between zero and 100%. Mosquito fish were not run every month because of availability. Effluent results met EPA criteria in three months. Control survival rate for all three months was inconsistent and in some cases had lower percentage survivability (Table 3).

Invertebrate Toxicity Testing

United States Environmental Protection Agency (1993; 1994) recommends that *Daphnia* bioassay results be considered acceptable only if control survival is at least 90% in the fours days of the test. *Daphnia* survival rates in the effluent water ranged from zero percent to 100%. September through December results met EPA criteria. Survival percentage rate in the control for *Daphnia* ranged from zero percent to 80% (Table 3).

DISCUSSION

The major points to be made concerning adhering with EPA toxicity testing protocols are low dissolved oxygen and high hydrogen sulfide levels in the summer and early fall months. Further, early on it was thought that chlorine in the control caused inferior survival results. After a feeding regime was began in June, 2005, survival in the control increased assuming then that chlorine was not solely responsible for mortality. Toxicity assays were not run until April due to a clogged return pipe carrying effluent.

Problems identified within the 2002 Annual Report were corrected. Eggs are obtained by the HSU Hatchery and funded through the city of Arcata on a quarterly basis. First, the need for fish for the Fish Physiology course, taught by Dr. Helen Mulligan, did not interfer with obtaining the proper age class fish needed to run the bioassay. Second, *G. affinis* were collected from either No Name Pond or Freshwater Lagoon and are currently reared at the wastewater aquaculture facility in hatchery water for future use. Third, hydrogen sulfide has been run every month Hach instrument was operational (Table 1) which has helped to concisely determine the cause of inadequate results. This year's results did not show an increase of *Daphnia* as did 2003 (Table 3). The lack increase of *Daphnia* in the wastewater test chambers may indicate that the wetland effluent too harsh due to hydrogen sulfide and low dissolved oxygen. It is believed that the increased mortality in the control is due to hydrogen sulfide solubilizing into the control water. The levels were so high that the student technician had to wear a respirator. This information should be used immediately to correct the hydrogen problem. In concert with the remedy, less chlorine would be necessary and thus, providing a savings to chlorine costs to the City.

Daphnia and fish were feed throughout the toxicity test. It has been determined that zero survival in the control has been due to starvation. The control contains no nutrients which made it impossible for the test organisms to survive which may explain the consistently low *Daphnia* survival rates seen in the control water. Temperature, dissolved oxygen and pH when measured were all within normal levels to support survival (Table 2). Therefore, it was thought that chlorine levels contributed to

mortalities but a new chlorine removal filtration unit was installed without any appreciable differences.

Rainbow trout high mortality rates were due to the extremely high hydrogen sulfide levels and/or temperature (Table 1). This explanation is supported by concomitant mosquito fish mortality results. Temperatures exceeded lethal levels for salmonids in both the effluent and control assays. Chlorine also reached unhealthy concentrations. The effluent dissolved oxygen concentrations were below minimum levels for salmonid survival. Some of the aberrant results stemmed from clogged tubing by the accumulation of biofilm. In conclusion, the combination of low dissolved oxygen concentrations, elevated temperatures, and heightened hydrogen sulfide levels prevented the survival of all organisms tested during most months.

It is important to perform bioassays on several different species and types of organisms to fully understand the quality of water they inhabit. The quality of water is poor. As temperature increased dissolved oxygen decreased, a situation that became sub-optimal for fish. Natural wetland processes result in the production of hydrogen sulfide by sulfate reducers under anaerobic conditions. Because this will be an ongoing problem naturally, perhaps aeration before discharge should be considered.

2005 INVENTORY[υ] FOR THE
WASTEWATER UTILIZATION PROGRAM
Humboldt State University

Meters/Probes/Supplies:

Wastewater Utilization Laboratory

- Gateway computer (WFB 264)
- Lanier photocopier [X*]
- Nitrate colorimeter
- 2 Hanna™ pH meters
- 2 Hanna pH probes
- YSI Salinity-Conductivity-Temperature Meter w/ 50 ft cord probe
- Dissolved oxygen meter
- Yellow Springs Instrument dissolved oxygen probe
- YSI dissolved oxygen meter ‡
- Sounder
- Compost thermistor
- Aquatic insect light traps (2)
- Challenger inflatable boat
- Refrigerator ‡
- Refrigerator - for BOD
- BOD bottles
- BOD Incutrol unit
- Incubator ‡
- HACH Chemical Oxygen Demand reactor ‡
- Stirrer/Hot plate ‡
- 2 Stirrer/Hot plate
- Vortex - ‡
- Vortex
- Ohaus scale
- Reotemp Stainless Steel Bi-Metal Thermometer 12'
- Xyron Pro 1250 Mounter (Poster presentations)
- Nitrate Color Disc Kit - low and high range HACH
- Nitrite Color Disc Kit - low range HACH
- Ammonia Color Disc Kit - 0-2,5 mg/L HACH
- HACH DR 890 hand held meter ‡
- HACH DR 890 hand held meter
- Salinity refractometer
- Multi-channel pipet
- Total solids crucibles
- Insect collecting equipment ‡
- Portable Anaerobic Chambers (12) ‡
- Sieve Set
- Secchi depth Aquascope II
- Secchi disk with weight sounder

- Kemmerer and messenger sampler
- Plankton net
- Seerite light box
- Dessicator with procelain plate
- 2 Face shields
- Labline Mistral Multi-mixer
- Fisherbrand Isotemp waterbath
- Whirlpool dishwasher
- Media flow pump
- Mosquito dipper sampler
- In-stream Flow Meter
- Centrifuge

Aquaculture Facility

- Submersible pump
- Vane set
- Gas power sprayer
- Refrigerator (Hatchery) ‡
- Heat pumps (4)
- Generator battery
- CO_2 Tank
- K24-N1 Fish Hauling Tank
- Picking Nets (6)
- Fry Nets (4)
- Bait Bucket Aerator
- Upright freezer *
- Fingerling Nets (4)
- Monorail Nets (2)
- Magnum Filter System
- OceanCleer Micron Filter
- Mag Drive Pumps
- 4 freshwater flow meters
- 1 salt water flow meter
- MacIntosh SE 30 computer/ Stylewriter printer‡
- 35 10-gallon aquaria
- Chiller
- Back-up generator
- Back-up blower
- Ohaus portable scale
- Circular saw
- Various tools: screw drivers, hammers, measuring tape, stainless steel shelving

Bioassay

- Challenger Aquarium Pump - MDA 22
- Sweetwater High Power Aerator Pump
- Overflow (600 GPH) for Large Aquaria (9)
- Bioassay Containers and Covers (2 gal.) (7)
- Large Aquaria (50 gal) (3)
- 4 valve spliter for aeration
- Sweetwater blower
- 2 gallon buckets (5)
- Diffusers (12)
- Chlorine Test Kit
- Fish nets (3)
- 10 gal. Aquaria (20)

Tracer (location: Environmental Engineering)

- Eppendorf pipetter (metals study)
- 12 v battery (1)
- 12 v pumps (2)

Books

- Standard Methods, 19th/20th Editions
- Insect Ecology
- Waterborne Diseases
- EPA Manual - Acute Toxicity of Effluents... (Bioassay)
- Environmental Chemistry, 7th Edition (Manahan)
- Wastewater Organisms

‡ = Brenneman's personal property
* = Donated by Andrew Jensen
υ= Most supply monies was spent on expendable materials. Added Gateway computer and Internet hookup and new Conductivity meter (to replace stolen meter)
X* = Donated by HSU Foundation but City grant pays for copies at a reduced rate

2005 ANNUAL REPORT

Arcata Wastewater Aquaculture Project Report

C.M. Jones and K.J. Brenneman
Wastewater Utilization Graduate Program
Department of Fisheries Biology
Humboldt State University

January 5, 2006

Employee List for 2005:

Dr. Kristine Brenneman
Program Leader

Nicholas Simpson

Co Manager

Cory Jones
Co Manager

Patrick Wiley
Hatchery Technician

Jared Kurtz
Hatchery Technician

Aryan Dilts
Hatchery Technician

Jason Soto
Hatchery Technician

Introduction:

2005 was a productive year for AWWAP. Lessons learned during the 2004 year were put to good use and positive results followed. Improvements were made to the summer ponds (SPs) in order to restrict otter predation. Further improvements to

AWWAP included the hatchery receiving a new coat of paint. Finally, we received a new air pump to serve as a backup for the pump that provides air to the yearling and summer ponds.

IACUCC conducted two inspections (3/24 and 10/11) resulting in satisfactory conditions on both occasions.

Tours:

Seventeen tours conducted by AWWAP employees were given to various interest groups. Each group brought new insight and appreciation for the project. See Appendix I for further details.

Facility improvements:

Improvements made to the aquaculture facility included a fresh paint job for the exterior of the hatchery, otter deterrents for the yearling (YP's) and summer ponds (SP's), and a new air pump for the ponds. See Appendix II for further details.

Projects:

There were a few continuing and new projects conducted during the 2005-year. In addition to the standard water quality testing and bioassay, metal content of the aquaculture ponds was studied. Continuing projects included growing mosquito fish and stickleback in dechlorinated wastewater effluent and monitoring the Jolly Giant Creek Fish Trap.

An assessment of the metal content of treated wastewater contained in the summer and yearling ponds was conducted in the spring of 2005. Metals of concern were

copper, lead, cadmium and zinc. All levels were determined using atomic absorption and colorimetric methods. Concentrations were well below toxic levels with the exception of cadmium, which requires further study. For more information on this study, refer to the Soto and Dilts report submitted to the city of Arcata in 2005.

Approximately 15 stickleback and mosquito fish were held from May of 2004 to February of 2005 in dechlorinated effluent without feeding and survived during this entire time period.

From 9/23/04 until spring of 2005, the Jolly Giant fish weir was in operation. Since 9/23/04, no adults had been trapped. Vandalism occurred three times with this trap. When the trap was initially put in, the upper weir structure was torn apart. This occurred the following week for a second time after the weir had been repaired. The third form of vandalism occurred when in late March, an eight square inch hole had been cut in the bottom of the trap, hidden from view.

The Jolly Giant Creek fish weir has been in operation again since October in order to intercept potential returning salmonids. No fish have returned as of this writing.

Salmonid rearing:

In early March, 2200 steelhead fry and 1200 coastal cutthroat fry were obtained from the Humboldt State University hatchery. Of these fish, 446 steelhead and 544 cutthroat survived. On September 9, these fingerlings were transferred to SP1 and SP2 respectively. As of this writing, all fish are alive and growing well in the wastewater ponds. Their diet of invertebrates is supplemented with Silvercup feed on a daily basis.

On October 25, approximately 1000 rainbow trout fry were obtained from the HSU hatchery. These fish are being reared in both wastewater and freshwater in

AWWAP's "fish barn". Although these fish will not be used for stocking, they are instrumental in providing research opportunities, such as bioassay.

A lack of a viable broodstock due to otter predation resulted in no fish being spawned at AWWAP in 2005. Fish now being grown in the summer ponds will serve as the future broodstock and will be ready for spawning in early 2007.

Conclusion:

Improvements made to the adult rearing ponds have proven successful in deterring otter predation. Consequently, salmonid broodstock production is proceeding with positive results.

Fish numbers in various age classes are approaching historical numbers for the Arcata Wastewater Aquaculture Project. In addition to the year-old broodstock in the summer ponds and the fingerlings in the fish barn, Cutthroat and Steelhead eggs will be obtained in early 2006 in order to get the hatchery up in operation once again. Having fish at a variety of stages is beneficial for educational opportunities such as tours, research, and other individual interests.

Goals for 2006 include maintaining fish numbers and proceeding with new and current research projects for which AWWAP is intended. A new wastewater utilization graduate student will begin studying the metal uptake of salmonids grown in wastewater.

APPENDIX I

Date	Tour	No.
3/1/05	Dr. Hendrickson's aquaculture class	8
3/13/05	Charlie Simpson	1
3/24/05	IACUCC Inspection	8

3/24/05	Oregon State University students	8
3/25/05	Dr. Gearheart's wastewater wetland class	10
4/13/05	Jang Lee (fisheries grad student)	1
7/9/05	Zach Wahl	1
7/14/05	Jason Ogawa	1
7/15/05	U of O Fish Camp	8
7/21/05	Birru Morgan	1
9/16/05	Mike Christie	1
10/11/05	IACUCC Inspection	4
10/11/05	INRSEP students	5
10/24/05	Misty Niemeyer	1
11/9/05	Paul Meyer	1
12/28/05	Lynette Featheringill	1
Fall 2005	Carol Lasko's Quantitative Analysis Class	

Table 1. Tours and inspections occurring at the Arcata Aquaculture Wastewater Project facility for 2005.

DATE (2005)	NAME OF GROUP OR INDIVIDUAL	#	COMMENTS
2/25	Mr. and Mrs. Kopple	2	Want to volunteer
3/03	Jerry (Last name unknown)	1	Sturgeon farmer from bay area
3/12	Terra with Heart of Humboldt	1	Filmed Dr. Brenneman for documentary

3/14	Steve and Ann Carmack	2	Gold Beach, Oregon
3/26	Fish Disease class	5	Dr. Hendrickson, HSU
3/27	Chuck Weiderhold	1	Mentor at Auburn Interpretive Center
4/02	Jennifer Simpson	1	Prospective student
4/10	Cutten Charter School	12	
4/20	HSU Aquaculture 370 class	20	Dr. Hendrickson, HSU
4/27	Tour to high school students	25	School not documented
5/11	Ray Moss	1	INRSEP student
5/27	IACUC	6	Inspection
5/24	Mike Wallace, Dept. of Fish and Game	1	79 cutthroat for REEF
6/10	Natural History Museum, HSU	14	12 children, 2 adults; fish experience
6/22	Robert and Spuz Mobley	2	Very pleased with facility tour
7/30	Mike Wallace, Dept. of Fish and Game	2	Brought daughter for tour
8/23	Mike Sandler	1	Clean Water Institute
9/08	Terry Roelofs, HSU and camera crew	3	Film documentary on George Allen
10/24	Leo Juarez and Phillip Ercolini	2	Tour
11/12	American River College class	25	
11/18	IACUC	7	Inspection
12/21	Steve Tyler and Bill Gilmer	2	Inspection of new lighting and roof

J. Kurtz and K. J. Brenneman
Wastewater Utilization Program
Department of Fisheries Biology
Humboldt State University

January 8, 2006

RESULTS AND DISCUSSION

Water quality parameters were recorded on a semi-weekly basis at the Arcata Wastewater Aquaculture Project. The seven outdoor ponds monitored were South Pond, Yearling Ponds (YP) 1, 2, 3, and Summer Ponds (SP) 1, 2, 3. Ponds were monitored for dissolved oxygen (mg/L), temperature (°C), pH, salinity (ppt), conductivity (µmhos/cm), ammonia (mg/L), nitrite (mg/L), and nitrate (mg/L).

Dissolved oxygen and temperature were recorded with a Yellow Springs Instrument (YSI) dissolved oxygen meter. A Hanna pHep meter was used for recording pH and conductivity. Salinity was recorded with a generic salt meter purchased from Nasco. Ammonia, nitrite, and nitrate concentrations were measured using Hach (Loveland, Colorado) methods.

Generally, South Pond and YP ponds stayed at approximately the same levels throughout the test period. However, an increase of precipitation during winter caused levels to vary slightly. Yearling pond 3 showed much higher levels of ammonia than the other ponds (Figures attached).

On multiple occasions, one and sometimes two otters were seen in YP1 and YP2 for extended periods of time. To ensure that the otters stayed out of SP1, and SP2, an electric fence around the perimeter was repaired. Summer pond 1 and 2 were also fortified more with fencing and rock dams to ensure the otters would not enter them.

Summer pond 2 at one point became overgrown with *Lemna* because salinity levels had dropped considerably. This was due to the salt pump going out. The City was notified and a near salt water pump was installed. In the meantime to

alleviate the *Lemna* problem, water from the South Pond was pumped into SP2 to increase the salinity level and to rid the plant material.

Summer pond 3 has no direct water connection and is not aerated. Ammonia was not found in the recordings. On one occasion a small amount of nitrite was detected.

Mosquito abundance in four constructed wastewater treatment wetlands in Arcata, California

Submitted by:
Nicholas Simpson and Kristine Brenneman
Humboldt State University
Waste Water Utilization Graduate Program

January 11, 2006

INTRODUCTION

Hauser, Allen, Gearheart and Aldergrove marshes have been monitored for the past two years for mosquito larvae. The 2003 and 2004 sampling was only qualitative, and could only give general patterns of mosquito populations. This year, intensive random sampling allowed for quantitative larval abundance patterns, and in turn a better forecasting tool for 2006.

The 2005 mosquito populations were expected to be high due to the high number of breeding pools after record winter rains. In California, West Nile Virus cases rose from 779 in 2004 to 910 in 2005. Fortunately, no West Nile Virus cases in humans were recorded in Humboldt County in 2005; however, two birds tested positive in McKinleyville early spring. Next year's forecast is expected to yield low mosquito numbers in California.

Site Selection

Twelve sites were selected for each wetland using a random number generator (Microsoft Windows XP Excel 2004). Stratified random sampling allowed for sites selected only within *Hydrocotyle* beds. Of the 12 sites selected, six sites occurred within 60 cm of the inner margin and six sites within 60 cm of the outer margin of the *Hydrocotyle* bed (Figures 1 through 4). Inner and outer delineations were based on accessibility to randomly selected sites.

Field Collection

In 2005 from May through September, all wetlands were visited every two weeks within a two-day period. At each site, three 1000 ml dip samples were individually condensed into a 0.5 mm mesh sieve and transferred to a Whirl-pack containing 70

percent ethanol. Water temperatures and dip samples were collected between 0900 and 1300.

Macroinvertebrate laboratory analysis

Sorting of insects took place every week from May through October. After allowing samples to settle for one week, the supernatant was poured off until there was approximately 100 ml of a condensed sample left. The sample was poured into a Petri dish with a 10 cm ruler attached to the bottom. Using forceps and a dissecting scope (LEICA), organisms were sorted and measured to the nearest millimeter. After all sorting had been completed for the year, organisms were identified to family, and when possible, species. Mosquito larvae were mounted on glass slides, using CMC-10 mounting media (Masters Company Inc.) and identified to species using a compound microscope (LEICA) and identifying keys by Meyer and Durso (1998).

RESULTS

Allen and Gearheart marshes contained the greatest amount of total and potential West Nile Virus vectoring mosquitoes, where abundances averaged greater than one larva per dip in July (Figures 13 and 14). These patterns were independent of temperature (Figures 6 and 8). Hauser Marsh had consistently the lowest abundances of mosquitoes (less than 0.5 per dip) throughout the year (Figure 13). Aldergrove Marsh was treated with BTI on July 6, 2005, which reduced total mosquitoes on July 12, while at the same time, other marsh populations increased two fold (Figure 13).

DISCUSSION

Each of the four wetlands displayed various dynamics within mosquito populations. It is important to analyze each wetland individually to determine the best time to apply BTI. The latter part of June would have been the better time to control for mosquitoes within Allen Marsh (Figure 6). For Gearheart Marsh, an early August application would allow non-vectoring mosquitoes to exist, while interrupting the upward trend of West Nile vectors (Figure 8). This interruption may allow *Culex particeps* to be the dominant species in Gearheart through fall (Figure 7). Mid June would be a better time to apply BTI to Hauser Marsh (Figure 10). The BTI application in Aldergrove on July 6, 2005 proved to keep West Nile Virus vectoring species low by decreasing the dominant vector *Culex territans* and allowing non-vector *Culiseta particeps* to dominate. I would recommend the same application for next year.

CONCLUSION

Arcata has a diverse population of mosquitoes. In other habitats such as floodwaters, phytotelmata, and man made containers, mosquito numbers and composition differ from constructed wastewater wetland habitats. Although average dip numbers in wetlands appear to yield lower mosquitoes than flooded tires or garbage cans, surface area needs to be accounted for. Mosquito harboring *Hydrocotyle* beds within wetlands contain surface area in the acres. By knowing peak times of the year for mosquito emergence, we can intervene with control methods and prevent a potential outbreak of disease.

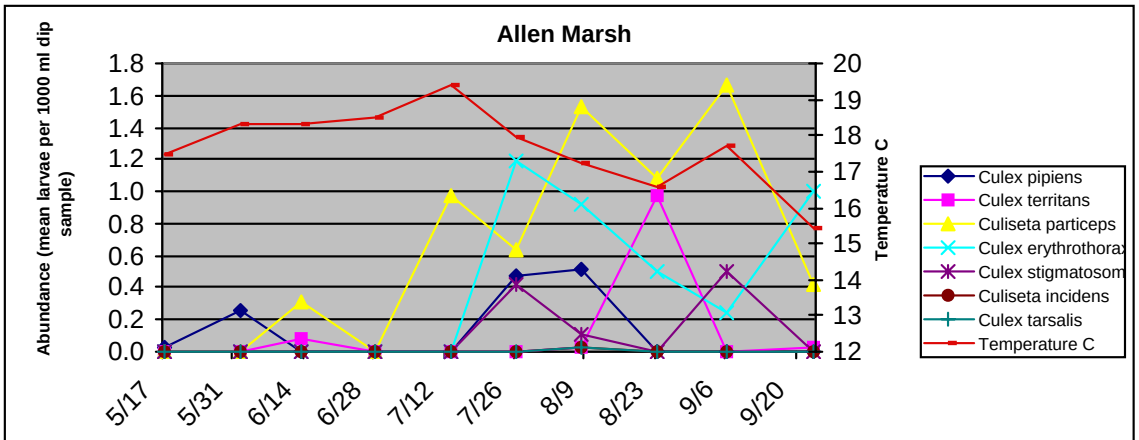
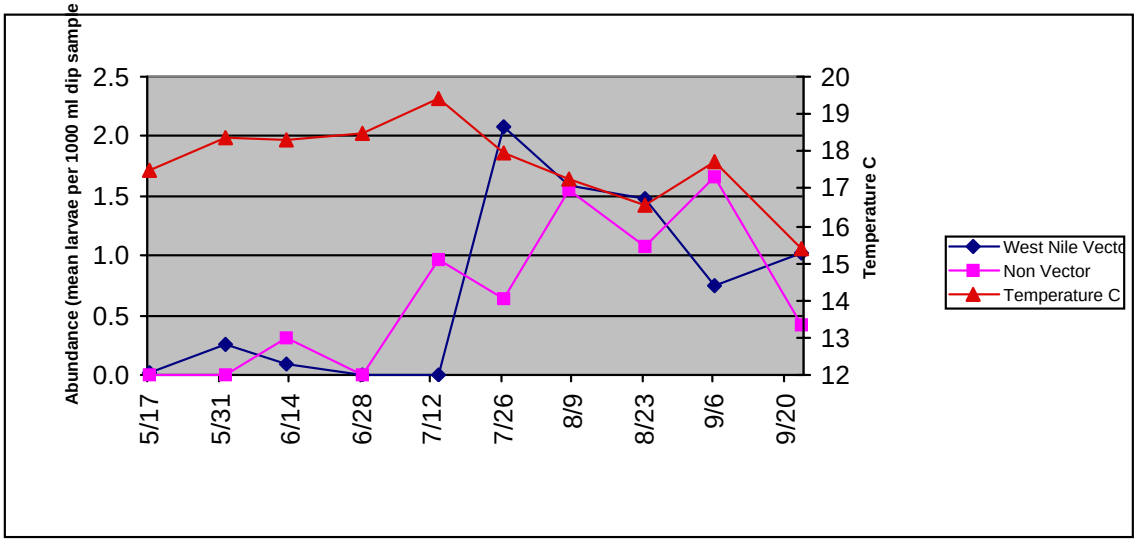
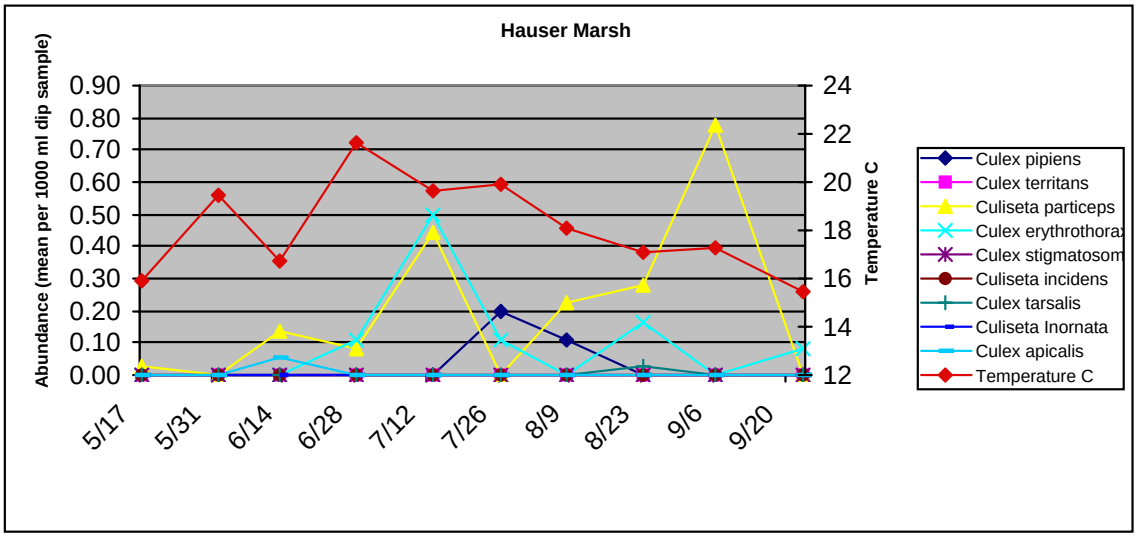


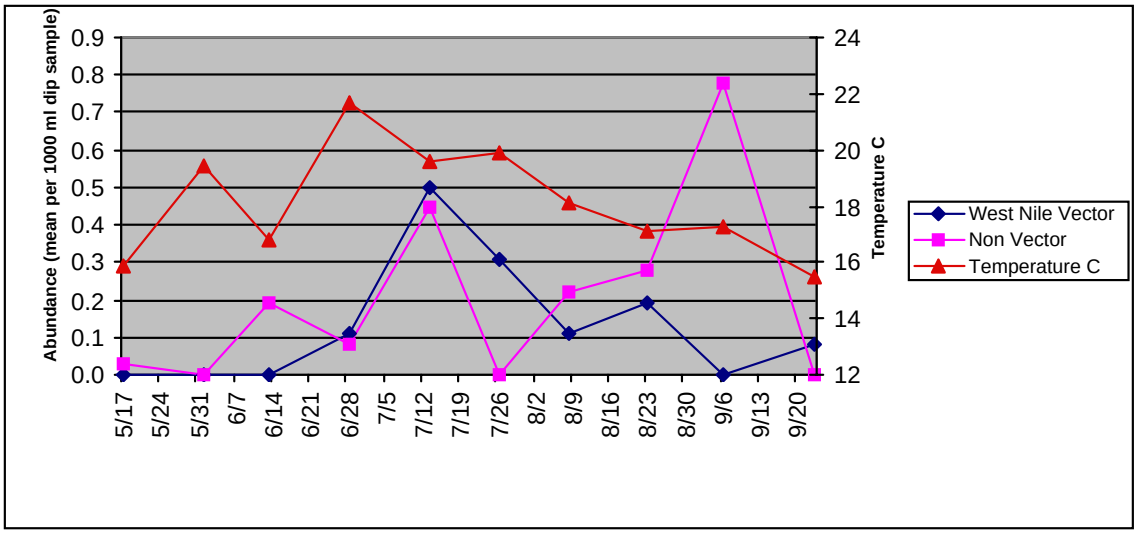
Fig. 5 Abundance of mosquito larvae in Allen Marsh in Arcata Ca during 2005.

Allen Marsh:





Hauser Marsh:



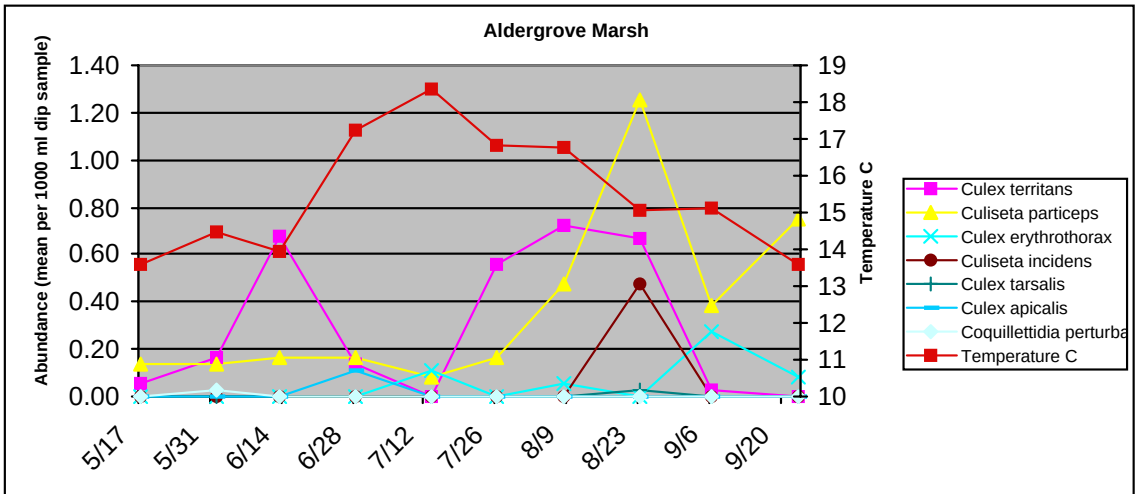
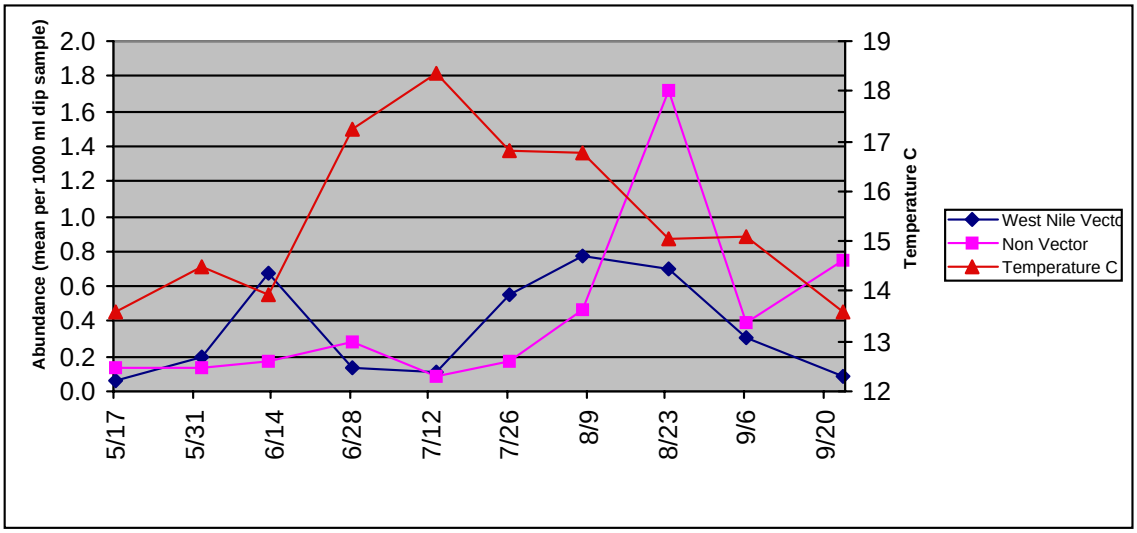


Fig. 11 Abundance of mosquito larvae in Aldergrove Marsh in Arcata Ca during 2005



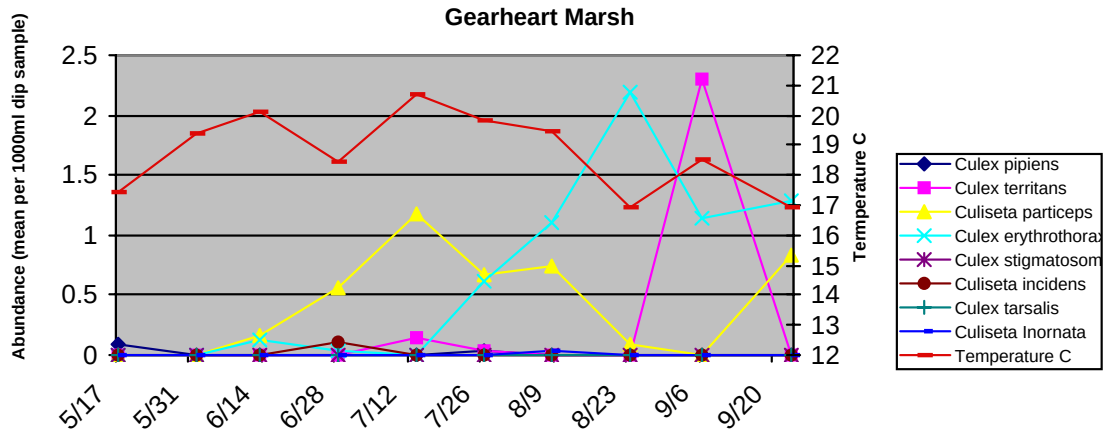
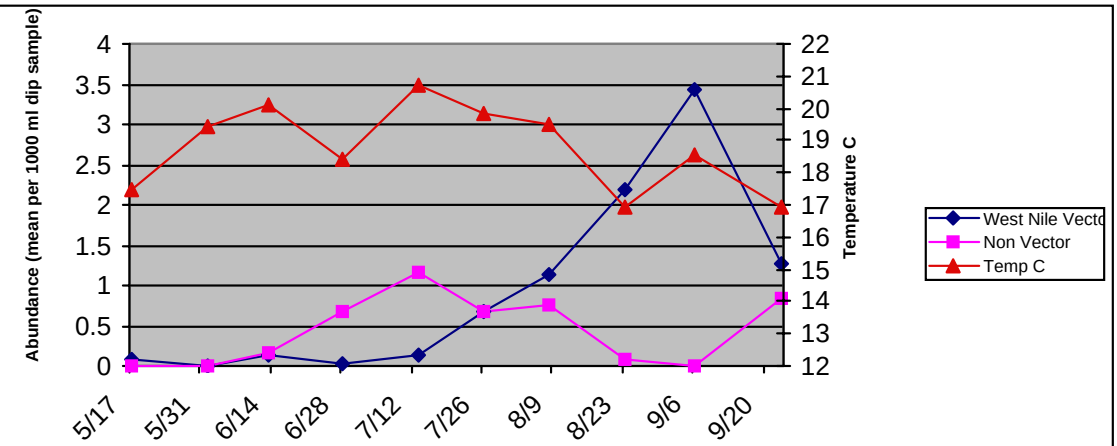


Fig. 7 Abundance of mosquito larvae in Gearheart Marsh in Arcata Ca during 2005.

Gearheart Marsh:



All Marshes:

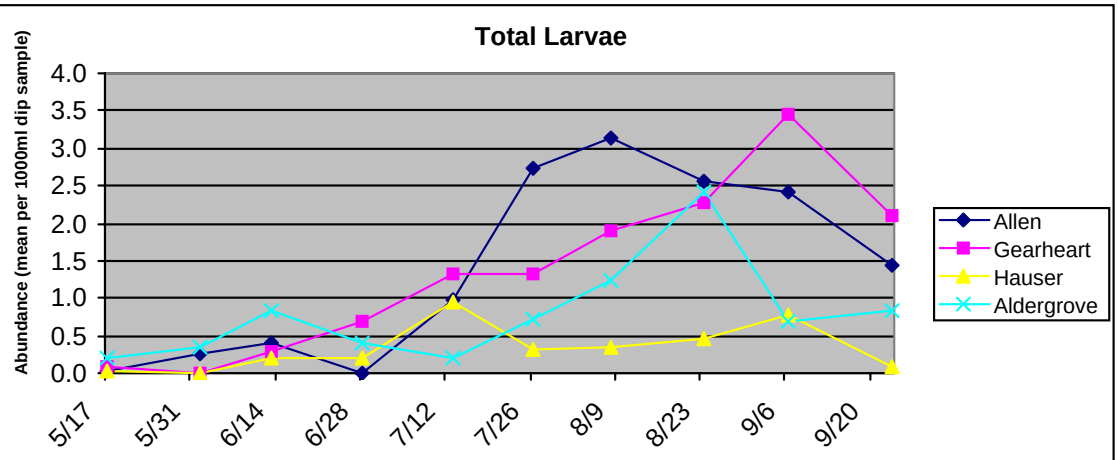


Fig. 13. Abundance of mosquito larvae in four constructed wetlands in Arcata Ca du

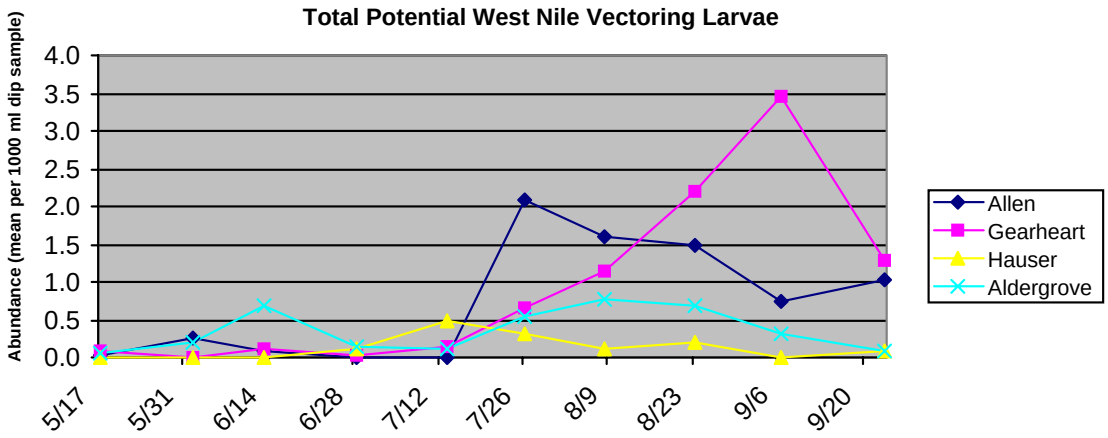


Fig. 14. Abundance of mosquito larvae in four constructed wetlands in Arcata Ca during 2005.