TERMINATION REPORT
SUMMARY

PROJECT TITLE: AQUARIUS v.3.0: Shellfish Sanitation Simulator and Analytical Software Incorporating Fecal Coliform, Rainfall and Tidal Activities

REPORT GIVEN IN YEAR: 2013

PROJECT WORK PERIOD: 4/01-2010_08/14-2013. Activities for year-1 funding of for the tidal-cycle work were not initiated until WRAC funds became available to the project in late December 2011. During the 2010-2011 periods, University of California funds were used to initiate work on a second sanitation model, *Pearl*, to be incorporated into *Aquarius III*. Initial work on the tidal-cycle component of *Aquarius III* began in February 2012 and will be completed in 2014.

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REASON for TERMINATION:
WRAC funds have expired. However, the project will be completed using additional funds from the University of California Davis (See project work period).

PROJECT OBJECTIVES:

(1) Develop version 3.0 of the Aquarius simulation and statistical analytical program that will synergize all the features of v.2.0, plus the inclusion of an integrated tidal program that will allow tide information to be incorporated into the formulas for assessing Adverse Conditions (Rainfall + Tide) in a shellfish growing area; (Note** A new analytical model, Pearl, has been added to the Aquarius 3.0 simulation program objectives)

(2) To provide sanitation information to the public, industry and regulatory agency representatives.

(3) Develop outreach materials including a manual of operations; two audio Podcasts describing the principles of the National Shellfish Sanitation Program and the classification of growing waters; and one Flash Video describing the operation of Aquarius v.3.0. (Audio podcasts may be replaced by additional Flash Videos).

PRINCIPAL ACCOMPLISHMENTS:

Background: Oysters, clams and mussels are filter-feeders that can concentrate potentially harmful constituents such as pathogens as they filter water to capture food. To protect the public from contaminated shellfish, the FDA and the Interstate Shellfish Sanitation Conference (ISSC) establish regulations for uniform application of shellfish regulations under the National Shellfish Sanitation Program (NSSP). The NSSP mandates that state shellfish authorities shut down shellfish harvest if growing area water quality drops below food safety levels. The NSSP uses an indicator group of bacteria, fecal coliform, to assess the potential that human pathogens may be present due to fecal contamination. Bacterial contaminations are expressed using the units of Most Probable Number per 100 milliliters (MPN/mL). The NSSP uses a "14/43" standard for fecal coliform concentration. This means that the fecal coliform median, or geometric mean, must not exceed 14 MPN/100 mL, the estimated 90^{th} percentile should not exceed 43 MPN/100 mL, and no more than 10 percent of fecal coliform samples taken may exceed 43 MPN/100 mL for a 5-tube test.

NSSP guidelines allow shellfish harvest when the estimated 90^{th} percentile of fecal coliform concentrations are below 43 MPN/100mL. Shellfish growing area classifications include "Approved" and "Conditional Approved." Approved areas meet the 14/43 standard year round. Areas classified as Conditionally Approved are closed temporarily based on things like rainfall events and river flow that can result in the water quality in harvesting areas not to meet the water quality standards. Under a Conditionally Approved rule, an area is open for shellfish harvest unless a predefined event occurs, at which time it is closed for harvest for a period that is predetermined by the regulatory agency.

At present, regulatory agencies use regression models (multiple regression or logistical regression) to calculate closure rule thresholds and durations. Using a regression analysis establishes a relationship between the response variable (observed fecal coliform concentration) and several predictor variables, such as observed rainfall, river flow, river stage, water temperature, salinity, and tidal stage. An EPA (U.S. EPA, 1999) covering regression models concludes that, …*"It is important to note that in most cases predictive tools are not used alone; they are usually combined with real-time monitoring of water quality conditions. These two processes are dependent on one another. The frequency of water quality sampling might be reduced in the future once a reliable modeling approach is in place…."* Aquarius contained the first non-regression, algorithm-driven computerized shellfish sanitation model developed for rapid evaluation of new rainfall closure rules for conditionally approved shellfish growing areas. Aquarius uses algorithms, which are a set of digitally-created rules that precisely defines a sequence of operations. The algorithms mimic exactly the procedural actions performed by regulatory agencies in determining fecal coliform, sanitation conditions in shellfish growing areas.

Accomplishments: In the development of Aquarius III, we incorporated the new statistical formulas developed for version II to perform shellfish sanitation, fecal coliform data analysis and calculate the upper limits of both the geometric mean and the estimated 90th percentile. These formulas augment the normal NSSP statistical methods used in dataset analysis and increase the overall sensitivity of the standard analysis of fecal coliform samples. All components of Aquarius II have been incorporated into Aquarius III.

As part of Aquarius III, we developed a new non-regression, computerized shellfish sanitation model (*Pearl*) capable of working in two modes that provide agencies and industry the ability to visualize and analyze fecal coliform observations in ways that permit a qualitative assessment of shellfish closure rule adequacy. The models are applicable to all watershed types that are influenced by rainfall, river stage, river height, or effects of tide. Aquarius III contains two bar graph functions: 1) Pearl's True Negative Values Bar Graph that illustrates the true negative values that appear to be operating under adequate harvest closure standards and, 2) Percent True Negative Index that denotes number of estimated 90^{th} percentile values appearing below the Pearl limit of 26 MPN/100 mL. Both are used to identify problem areas in growing areas that might require closure rule adjustment.

We also built new algorithms that incorporate the Pearl model into Aquarius III. In stand-alone mode, Pearl performs a multi-year analysis using fecal coliform concentration data from shellfish growing areas (viewed in scattergrams) illustrating when and at what magnitude sanitation problems occurred in growing areas. New algorithms provide a feed-back loop within Aquarius III, thus micro-adjustments to shellfish growing area closure rules that are made by Aquarius can be studied in Pearl's scattergrams, and re-adjusted in Aquarius thus optimizing the closure threshold and duration. These adjustments can be made in less than two minutes.

During this grant cycle, we ground-tested the original Aquarius II model concepts on Arcata' Bay's California sanitation database and published the model and results of the study (Conte and Ahmadi, 2011) We also ground-tested the Pearl model on Oakland Bay Washington's sanitation database, and the sanitation databases of seven shellfish bays in Texas, published the Washington study and submitted the manuscript for the Texas study (Conte and Ahmadi, 2012; Conte and Ahmadi, submitted). During the peer-review process, both the Aquarius and Pearl were scrutinized by sanitation engineers and modelers without a single challenge presented regarding the concepts or formulas used in the models.

Using real sanitation data we established Pearl Limit's for geometric means and estimated 90^{th} percentile (8 MPN/100mL and 26 MPN/100mL respectively) for a 5-tube Test. While applying the Pearl Model to sanitation databases of California, Washington and Texas; we discovered that state regulatory agencies are inadvertently applying the Pearl Limits in their closure rules and not the NSSP standard of 14/43 MPN/100 mL (Conte and Ahmadi, 2012; Conte and Ahmadi, submitted). Preliminary data analysis of Florida sanitation datasets show the same results (Conte and Ahmadi, unpublished). This consistent revelation in different regions of the country allowed us to challenge the existing NSSP 14/43 standard and propose a new 8/26 MPM/100 mL standard (Conte and Ahmadi, 2012; Conte and Ahmadi, submitted).

We have identified a computer tide program that will be incorporated into Aquarius III that will be used in worst-case scenario analyses related to potential closure rule changes and in general sanitation data analysis. We are currently constructing algorithms to incorporate the tide program elements into the Aquarius and Pearl models of Aquarius III, and expect the tide component to be a significant and powerful analytical tool that will complement the analytical ability of the pearl model. We are finishing the construction of drop-down menus for Aquarius III. Construction has begun on outreach products, including the digital Articulate presentations and an Aquarius III software manual.

IMPACTS

Issue: State health agencies are responsible for overseeing shellfish harvest to ensure that shellfish are safe for human consumption. When rainfall washes fecal coliform into shellfish growing areas, shellfish harvesting often ceases which results in loss of revenue. Agency tools used to analyze sanitation conditions and modify closure rules for shellfish growing areas are time-consuming and expensive in terms of person hours necessary to implement, thus results in fewer studies conducted and modifications implemented.

Response: A new sanitation model (*Aquarius 2.0*) was developed, which to process rainfall and fecal coliform data and adjust harvest closure rules. This project developed *Aquarius 3.0* which adds a second model *Pearl* to the *Aquarius* model, and is adding a unique tidal component designed to evaluate and manage growing areas; and provide a more sensitive and accurate analysis of sanitation conditions in shellfish production bays. *Aquarius 3* incorporates a feedback loop between the models that allows analysis, adjustment of closure rules, and re-analysis of the data to evaluate the safety of such adjustments.

- The *Aquarius III* model (*Aquarius* and *Pearl*) provide agencies and industry the ability to visualize in both time and fecal coliform concentrations, and analyze fecal coliform observations, in ways that permit a qualitative assessment of shellfish closure rule adequacy and sanitation conditions in shellfish growing areas. The models are applicable to all watershed types that are influenced by rainfall, river stage, river height, or effects of tide.

- Allows rapid examination of large data sets and reduces human error.

- Adds new sensitivity to standard NSSP statistical analyses through additional equations that calculate the upper limits of the geometric mean and upper limits of the estimated 90th percentile of fecal coliform concentrations.

- Provides greater confidence in the decision making process through increased sensitivity of analytical methods provided by new analytical equations.

- Provides cost savings for regulatory agencies by shortening the person-hours associated with shellfish growing area analysis.

- Provides potential profits to the shellfish industry by potentially opening more harvest days that might otherwise be closed, and with no increased risk of illness to shellfish consumers.

- The *Aquarius* model, working in tandem with the *Pearl* model, provides a mechanism to analyze fecal coliform concentrations in shellfish growing areas over time, make micro-adjustments to shellfish growing area closure rules, re-examine the results of closure rule changes, and maximize days open to harvest and still protect the public from consuming contaminated shellfish.

- Addition of the new *Pearl* model to *Aquarius* 3.0 revealed that states inadvertently employ the Pearl Limits of 8 MPM/100 mL for geometric mean and 26 MPN/100 mL for estimated 90th percentile in their closure rules and not the NSSP standard of 14/43 MPN/100 mL; and thus allows the authors to challenge the NSSP Standard and recommend replacing it with a new 8/26 MPN/100 mL for a 5-tube test.

RECOMMEMDED FOLLOW-UP ACTIVITIES

Follow-up activities will include completion of algorithms, drop-down menus, and software program that will incorporate all elements of Aquarius II into Aquarius III. This will include the reconfiguration of the Aquarius and Pearl model algorithms to include the tidal element in the sanitation analyses. We will also complete the software manual and outreach *Articulate* presentations. This should be completed in 2014.

We will continue to analyze additional state shellfish sanitation databases and submit resultant manuscripts to peer-reviewed journals. The next set of databases to be analyzed have been obtained from regulatory agencies in Florida, Mississippi, Georgia, South Carolina, North Carolina and Virginia.

SUPPORT:

YEAR	WRAC-USDA Funding	OTHER SUPPORT					Total Support
		University	Industry	Other Federal	Other	Total	
2010	$6,000	$6,000	-	-	-	$12,000	$12,000
2011	$6,000	$2,000	-	-	-	$8,000	$8,000
2012	$8,000	$2,000	-	-	-	$10,000	$10,000
TOTAL	$20,000	$10,000	-	-	-	**$30,000**	**$30,000**

Note: All of the funding for the Aquarius project was directed to pay for a portion of the salary of Dr. Abbas Ahmadi, Senior Programmer, Department of Animal Science, University of California, Davis.

SUBMITTED BY: _____ _____
Title: (Work Group Chair or PI) Date

APPROVED: _____ _____
Project Monitor Date

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PUBLICATIONS, MANUSCRIPTS, & PAPERS PRESENTED:

Papers:

Conte, F.S. and A. Ahmadi. 2011. A computerized model for evaluating new rainfall closure rules for conditionally approved shellfish growing areas. Transactions of the American Society of Agricultural and Biological Engineers, 54(3): 909-914. 2011

Conte, F.S. and A. Ahmadi. 2012. Evaluation of Fecal Coliform Samples Collected from Oakland Bay, Washington Using a New Model for Estimating the Ninetieth Percentile Criteria for Evaluating Shellfish Growing Water. Journal of Water Resources Planning and Management, 75(3): 16-22.

Manuscripts submitted:

Conte, F.S. and A. Ahmadi. (Submitted). Application of the *Pearl* model to analyze fecal coliform data from conditionally approved shellfish harvest areas in seven Texas bays.

Presentations:

Conte, F. S. and A. Ahmadi. AQUARIUS: A Decision Support System for Aquaculture. 21st Century Watershed Technology: Improving Water Quality and Environment Conference Proceedings, Universidad EARTH, Guácimo, Limón, Costa Rica. 21-24 February 2010.

Conte, F.S. and A. Ahmadi. Evaluation of fecal coliform samples from Pensacola Bay, Florida (USA) using *Pearl* sanitation model. World Conference on Computers in Agriculture (WCCA 2012), National Training Institute of Farmers' Organization. Taipei, Taiwan. September 3-6, 2012.

Workshop and presentations presented:

Aquarius: *Pearl* Trend Analysis of Fecal Coliform Samples for Arcata Bay, California. Conte, F.S. and A. Ahmadi. Washington Shellfish Growers Conference, March 7 and 8th, 2011.

Aquarius: *Pearl* Trend Analysis of Fecal Coliform Samples for Oakland Bay, Washington. Conte, F.S. and A. Ahmadi. Washington Shellfish Growers Conference, March 7 and 8th, 2011.

Aquarius: A Computer Model for Optimizing Rainfall Closure Rules for Shellfish Growing Areas. Conte, F.S. and A. Ahmadi. Washington Shellfish Growers Conference, March 7 and 8th, 2011.

Tide Module: Aquarius. Conte, F.S. and A. Ahmadi. Washington Shellfish Growers Conference, March 7 and 8th, 2011.