TERMINATION REPORT
DETAILED

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. Note* 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 Note, 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 90th 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 90th 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 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. In the reconfiguration of Aquarius III, we incorporated the new statistical formulas developed for Aquarius version II into the *Pearl* model 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.

**Pearl's Structure and Algorithm:** The *Pearl* model is based on the NSSP recommended calculation methods. *Pearl* organizes the fecal coliform samples from each station into sequential, continuous groups, each consisting of 30 samples, as recommended by the NSSP. The first group includes the first thirty samples. The second group begins with the second sample and adds the following 29 samples (samples 2 through 31). This data grouping procedure is repeated for all observations. The fecal coliform dataset is divided into three subsets: (1) Open subset consisting of samples collected when the shellfish growing area was open for harvest; (2) Closed subset consisting of samples collected when the shellfish growing area was closed to harvest; (3) Combined subset consisting of both the open and closed subsets. *Pearl* then calculates four statistics (geometric mean, estimated 90th percentile, upper limit of geometric mean, and upper limit of estimated 90th percentile) for each data groups in the open, closed, and combined datasets using equations illustrated below and described by Conte and Ahmadi (2012) and Conte and Ahmadi (2012 Submitted).

a) Arithmetic mean and standard deviation of the logarithms (base 10) of the fecal coliform concentrations:

\[
\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n} 
\]

(Equation 1)

\[
s = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}}
\]

(Equation 2)

a) Calculate geometric mean by:

= \text{anti log}(\bar{x})

(Equation 3)

b) Estimated 90th percentile:

= \text{anti log}(\bar{x} + sd \times 1.28)

(Equation 4)

c) Upper limit of geometric mean at \( \alpha \) significance level:

= \text{anti log}\left(\bar{x} + \frac{sd}{\sqrt{n}} \times t_{\alpha, (n-1)df}\right)

(Equation 5)

d) Calculate the upper limit of estimated 90th percentile at \( \alpha \) significance level by:

= \text{anti log}\left(\bar{x} + \frac{1}{\chi^2_{\alpha, (n-1)df}} \times sd \times t_{0.10, (n-1)df}\right)

(Equation 6)
Where \( x_i \) denotes fecal coliform concentration converted to logarithm base 10, \( \bar{x} \) denotes the sample estimate of arithmetic mean, \( sd \) denotes the sample estimate of standard deviation, \( n \) denotes the sample size, \( \alpha \) denotes the significance level, \( t \) denotes the t-distribution, and \( df \) denotes the degree of freedom. The term \( t_{\alpha,(n-1)df} \) is calculated using the Excel function \( \text{tinv}(2*\alpha,n-1) \). The term \( \chi^2_{1-\alpha,(n-1)df} \) is calculated using the Excel function \( \text{chitinv}(1-\alpha,n-1) \). The value 1.28 in equation (4) is obtained from the standard normal distribution and is equal to \( z_{\alpha=0.10} \) for one-sided test.

Equations 3 and 4 correspond to the NSSP guidelines and are used for compliance with shellfish water quality standards by regulatory agencies (CDPH, 2006). Equations 5 (Mann, 1998) and 6 (Sheskin, 2007 and Snedecor and Cochran, 1967) calculate two additional parameters not used by NSSP: the upper limit of the geometric mean and the upper limit of the estimated 90th percentile.

**Diagnostic Tests and Decision Parameters Described as Follows:**

1) **Diagnostic Test** - The NSSP recommends the estimated 90th percentile of fecal coliform samples as a diagnostic test to screen growing areas for the presence of concentrations of the indicator organism, fecal coliforms, which represent the population of potential harmful pathogens due to fecal contamination. The growing area is closed to harvest when the estimated 90th percentile of fecal coliform samples exceeds 43 MPN/100 mL.

2) **Outcome of the NSSP Diagnostic Test:** The NSSP test outcome can be positive (= contaminated: closed) indicating that fecal coliform concentrations in the growing area are above the NSSP limit, or negative (= clear: open) indicating that fecal coliform concentrations in the growing area are below the NSSP limit. *Pearl* provides a more accurate and sensitive test to determine the true status of the growing area by performing Step 3 as follows:

3) **True Status of the Growing Area** - Each growing area tested either possesses, or does not possess the potential harmful concentrations of pathogens that are represented by the fecal coliform concentration. The NSSP test result (contaminated or clear) may or may not match the growing area's actual health status. In *Pearl*, we use a more sensitive test, the upper limit of estimated 90th percentile of fecal coliform concentrations, as an indicator of the growing area's actual health status as it incorporates the effect of sample size. If this parameter is below the NSSP limit of 43 MPN/100 ml, then it more accurately indicates that the actual health status of the growing area is "clear"; otherwise, the area is potentially contaminated.

4) **Calculating the "Pearl Limit" by Pearl:** *Pearl* sorts the estimated 90th percentile values of fecal coliform concentrations and the upper limits generated in step 3 from the largest to smallest. It then uses the user-specified NSSP limit (i.e., 43MPN/100mL for a 5-tube test) to search the sorted 90th percentile values for the first record where the upper limit is less than, or equal to the user-specified NSSP limit. Once that record is found, *Pearl* identifies the estimated 90th percentile for this record as the "Pearl Limit", which in our example is 26 MPN/100 mL for a 5-tube test. This means that the estimated 90th percentile of all the following records will be equal to, or less than 26 MPN/100 mL.
5) Comparing the Outcome of the NSSP Diagnostic Test with the True Status of the Growing Area - The comparison of the outcome of the NSSP diagnostic test with the true status of the growing area, indicated by Pearl's new parameter (upper limit of the estimated 90th percentile), generates three diagnostic zones, which are displayed in output scattergrams:

**True Negatives Zone:** A "clear" growing area is correctly diagnosed by the NSSP test as "clear". The estimated 90th percentile values appear below the Pearl Limit of 26 MPN/100 mL. In this case, the upper limit of the estimated 90th percentile values appears below the NSSP limit of 43 MPN/100 mL.

**False Negative Zone:** A "contaminated" growing area is incorrectly diagnosed by the NSSP test as "clear". The estimated 90th percentile values appear between the NSSP limit of 43 MPN/100 mL and the Pearl Limit of 26 MPN/100 mL. In this case, the upper limit of the estimated 90th percentile values appears above the NSSP limit of 43 MPN/100 mL.

**True Positive Zone:** A "contaminated" growing area is correctly diagnosed by the NSSP test as "contaminated". Both the estimated 90th percentile values and the upper limit of the estimated 90th percentile values appear above the NSSP limit of 43 MPN/100 mL.

5) Generating Output Graphs by Pearl: Pearl reads the list of estimated 90th percentile values of fecal coliform concentration, and their upper limits, as a first step in the production of scattergram (Figure 1).

In the generation of the output scattergram illustrated above, Pearl examines the list from the beginning date to the ending date, and for each entry in the list it checks if the estimated 90th percentile value is less than the NSSP Limit (i.e., 43 MPN/100 mL). If the estimated 90th percentile value is greater than, or equal to the NSSP limit, Pearl plots this value above the NSSP limit in the True Negative zone. If the estimated 90th percentile value is less than the NSSP limit, Pearl checks to determine if the value is below the Pearl Limit (i.e., 26 MPN/100 mL). If it is not less than the Pearl Limit, Pearl plots the value above the Pearl Limit, and below the NSSP limit in the False Negative zone. If the estimated 90th percentile value is less than the "Pearl Limit", Pearl plots the value below the Pearl Limit in the True Negative zone. Pearl continues plotting the values until the end of the dataset is reached. Pearl then checks if all the estimated 90th percentile values are below the Pearl Limit and in the True Negative zone. If so, the recommendation provided by the model is that the harvested shellfish do not pose any risk to shellfish consumers and the closure rule can be relaxed. If any of the estimated 90th percentile values are above the Pearl Limit, in the False Negative zone, or in the True Positive zone, then the recommendation provided by the model is that the harvested shellfish do pose a possible risk to shellfish consumers and the closure rule can be tightened.

Aquarius III also contains two bar graph functions: 1) Pearl's True Negative Values Bar Graph in figure 2 illustrates the true negative values that appear to be operating under adequate harvest closure standards and, 2) and Pearl's Percent True Negative Index, figure 3, that denotes number of estimated 90th 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 (Figure 2 and Figure 3 appear in the Appendix).
True Negative Values of 100 % (figure 2) indicate that these areas are safe for harvest. Values less than 100 % indicate sanitation problems; and the lower the value, the greater the sanitation problem.

The Pearl Index (Figure 3) is a value between 1 and 3. A value of 1 indicates that all the Estimated 90th Percentile values of fecal coliform concentrations are under the Pearl Limit of 26 MPN/100 mL (True Negative Zone: Harvesting shellfish is safe). A value greater than 1 but less than 2 indicates that the Estimated 90th Percentile values of fecal coliform concentrations are above the Pearl Limit but below the NSSP limit of 43 MPN/100 mL (False Negative Zone: Harvesting shellfish is NOT safe, although allowed by NSSP standards). A value greater than 2 indicates that the Estimated 90th Percentile values of fecal coliform concentrations are above the NSSP limit of 43 MPN/100 mL (True Positive Zone: Harvesting shellfish is NOT safe).

We have also built new algorithms that fully integrate the Pearl model seamlessly into Aquarius III. However, the Pearl model can also be used in a stand-alone mode in which Pearl performs a multi-year analysis using fecal coliform concentration data from shellfish growing areas. An analysis, such as viewed in the scattergram illustrated above, shows when and at what magnitude sanitation problems occurred in growing areas. The new integrative algorithms also provide a feed-back loop within Aquarius III, thus the results of micro-adjustments to shellfish growing area closure rules that are made by Aquarius can be studied in Pearl’s scattergrams, and then re-adjusted in Aquarius, thus optimizing the closure rule’s threshold and duration. Such micro-adjustments in closure rules can be made in less than two minutes.

During the latter part of the grant, we again 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 Washington’s agency, Oakland Bay sanitation database, and the Texas state agency’s sanitation databases of seven shellfish bays. We published the Washington study and have 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 model were scrutinized by sanitation engineers and modelers without a single challenge presented regarding the concepts or formulas used in the models.

Using sanitation datasets from state agencies, we established Pearl Limit’s for geometric means and estimated 90th percentile (8 MPN/100mL and 26 MPN/100mL respectively) for a 5-tube Test. Then, 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 MPN/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.

**RECOMMENDED 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 has been obtained from regulatory agencies in Florida, Mississippi, Georgia, South Carolina, North Carolina and Virginia.

**SUPPORT:**

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

Figure 1. Example scattergram showing the Estimated 90th Percentile of fecal coliform concentration in MPN/100 mL during open periods in Pensacola Bay, Florida. Each individual X in the scattergram represents 30 samples as recommended by the NSSP and described in Pearl's Structure and Algorithm on page 3 of this report.

Figure 2. Pearl's True Negative Values Bar Graph for fecal coliform concentrations during open periods in Galveston, Trinity, Matagorda, Carancahua, Lavaca, Tres Palacios, and San Antonio bays, Texas, United States, from 4-30-01 to 12-9-10.
Figure 3. Pearl Percent True Negative Index for fecal coliform concentrations during open periods in Galveston, Trinity, Matagorda, Carancahua, Lavaca, Tres Palacios, and San Antonio bays, Texas, United States, from 4-30-01 to 12-9-10.
PUBLICATIONS, MANUSCRIPTS, & PAPERS PRESENTED:

Papers:


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:


Workshop and presentations presented:


