Aquarius Version 2.0

Simulation Software for Rainfall and Water Quality Closure Rules
Designed for Agencies and the Shellfish Industry
Version 2009

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AQUARIUS

Version 2.0

Shellfish Sanitation Simulator

And Analytical Software

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AQUARIUS SOFTWARE MANUAL

ACKNOWLEDGMENTS

The Aquarius simulation and statistical software was developed by personnel of the Department of Animal Science at the University of California Davis as a decision tool for making changes in rainfall closure rules for conditional approved shellfish growing areas. The original effort was a cooperative project with personnel of the Shellfish Sanitation Branch of the California Department of Health Service (CDHS) and representatives of the California shellfish industry.

Upon completion of Aquarius version 1.0 (Conte and Ahmadi, 2003), the program was demonstrated at meetings of the Pacific Rim Shellfish Sanitation Conference and shellfish meetings held in California, Washington and Canada. State and Federal shellfish regulators expressed enthusiasm for the program, and identified twelve additional components that they considered essential to a fully mature program that could be used as a decision-making tool in their processes for initiating changes in rainfall closure rules.

To help defray a portion of the cost of producing Aquarius version 2.0, a rapid response grant was obtained from the USDA funded, Western Regional Aquaculture Center (WRAC). A core development team including University and CDHS personnel was formed; the University responsibility was to build the program and the agency responsibility to Beta-test the program using real data, cross check the accuracy of the program against their own statistical analysis, and to provide suggestions for the improvement of the program.

Special thanks are extended to both Development Committees, whose input, guidance and cooperation were essential to the development of the original concept of the Aquarius version 1.0 program, and the development of Aquarius version 2.0. .

Aquarius Development Committee

California Department of Health Services:

- Gregg Langlois, Senior Environmental Scientist
- Peter Krottje, Ph.D., Environmental Scientist
- Marc Commandatore, Environmental Scientist
- Glenn Takeoka, Chief, Environmental Health Services Section
- Ray Tom, Staff Environmental Scientist
- Jill Baltan, Environmental Scientist
- Jack McGerk (retired), Chief, Environmental Management Branch

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Introduction and General Description

Aquarius is a software simulation and statistical analytical tool designed to develop or modify rainfall closure rules that affect when filter feeding shellfish may be harvested from conditionally approved growout areas. Rainfall closure rules are established by sanitary surveys taken by Federal and State health service agencies during worst-case scenarios (storm conditions). They are based on measurements of the amount of fecal coliform bacteria washing into the bay, and how long it takes for the bacteria to disappear due to tidal flushing and current. Fecal coliform bacteria are used as indicator organisms for the presence of sewage and animal waste containing potential pathogens contaminating the bay. Filter feeding shellfish can concentrate the indicator organisms and the potential pathogens, the latter which form a health risk.

The National Shellfish Sanitation Program (NSSP) has established bacteriological standards for the classification of shellfish harvest areas. The analysis for fecal coliform takes 24 hours, and numbers of bacteria are expressed in the units of Most Probable Number (MPN) per 100 milliliters (ml). In the establishment of regulations governing harvest area conditions, statistical analyses are used to determine the level of accumulated fecal coliform bacteria as above or below a cutoff point of a most probable number (MPN) of 43. For areas to be classified as Approved, or Conditionally Approved, the level of fecal coliform in sub-surface water samples, at harvest, must meet the “NSSP 14/43” standard. The fecal coliform median or geometric mean must not exceed 14 MPN/100 ml, and the estimated 90th percentile may not exceed 43 MPN/100 ml.

Approved growing areas are areas in which the MPN is less than 43 (< 43), even during worst case scenarios (storms). Conditional Approved areas are growing areas that are subject to open and closed harvest periods based on accumulative rainfall. In Conditional Approved areas, data taken during the sanitary survey, and based on the accumulation of fecal coliform following a given level of rainfall, are used to establish closure timeframes for the harvest of shellfish. The area is reopened based on timeframes needed for flushing of the bay, usually by tidal exchange, until fecal coliform levels decline to levels < 43.

Because of the expense associated with continuous monitoring of water quality and shellfish meat, conditional growing areas are operated under a set of rainfall closure rules established by the regulatory agencies. The rainfall closure rules usually stay in place until a new sanitary survey is conducted. Sanitary surveys are conducted about ever 10 to 12 years.

Both the industry and State and Federal health service agencies would benefit from more frequent analysis of the bay that would be conducted in the interim between sanitary surveys. Often, during the period between full sanitary surveys, conditions in the watershed may improve or degrade with respect to sources of fecal coliform. If the watershed is improved, conditions may be such that a rainfall

closure rule would than be too strict and a shellfish company loses income because of excessive harvest closure. If the watershed degrades, a rule may be too lenient, and public health is at risk. However, more frequent full sanitary surveys are almost cost prohibitive, both monetarily and relative to personnel time commitment needed to process and evaluate data.

If there is evidence that bay conditions have improved, or degraded between major sanitary surveys, the State regulatory agency may work cooperative with the shellfish industry to obtain water and meat samples to make interim adjustments to the water quality closure rules. Even if the sampling cost are primarily financed by the industry, the restraints to the regulatory agency implementing such interim analysis are the cost in person power to run the multiple statistical analysis necessary to test numerous hypothetical rainfall adjustments and variations in closure periods. To overcome these constraints, personnel from the University of California Davis established a development team consisting of personnel from the California Department of Health Service and a representative of the shellfish industry to develop a tool (Aquarius) that could use shellfish sanitation laboratory and rainfall data to rapidly simulate hypothetical changes to existing rainfall closure rules and run comparative statistical analysis to determine the validity and safety of a new proposed rule.

General Description:

Aquarius version 2.0 is a expansion of the original Aquarius 1.0 decision making program (Conte and Ahmadi, 2003) developed for State and Federal health service agencies and the commercial shellfish industry. It is a Windows-based software simulation program, coupled with statistical programs designed to evaluate proposed or hypothetical changes in rainfall closure rules for conditional approved shellfish harvest areas. The program operates using rainfall and fecal coliform databases maintained by the associated regulatory agency. Aquarius is designed with an open architecture: meaning that it uses elements of Excel spreadsheet, Access database, Notepad, SAS Statistical Program, and Visual Data Basic Programming.

Version 2.0 operates within the same framework as its version 1.0 predecessor. The major upgrades in the new version includes an expansion on the options available to the user, improved data filtration options, and the inclusion of additional statistical analyses and parameters that increase the reliability of the decision making process to assure public health objectives.

In Version 2.0, the rainfall databases are in General Comma Separated Values (CSV) format, and can be altered by the agency as new data are added. Rainfall data can show cumulative daily, 6-hourly, Hourly and Tip-data format. When a rainfall database is loaded for a simulation, it may be viewed in a browse mode, but not altered. The data is protected. However, the database may be exported

to an Excel spreadsheet to correct an error, or to run a separate analysis on the data. Another rainfall database can be loaded into the program, and recalculated to establish the cumulative rainfall calculations.

Fecal coliform data is maintained and operated under the same conditions, with both archived data that can be protected, but browsed or exported when activated by the software. Exporting or browsing the databases does not alter the original, protected databases. The authors recommend that health agencies protect the integrity of the agencies' official archived data. Although industry can analyze various scenarios when proposing changes, the decisions are made by the agency that re-runs the proposed scenarios using their own archived databases. This allows the responsible agency to protect the integrity of the database when the program is operated in a public forum.

When performing an analysis, the pertinent rainfall and fecal coliform databases are individually loaded from archived files into the software program before initiating the simulation and statistical analysis. The first data input window shows the identification of the Region, up to a combination of four Sites, Site dates, and up to a combination of four Sample Types (databases). There is an option for a Three-Tube Test, Five-Tube Test, 12-Tube Test, Membrane Filter (MF); and Restricted 3-, 5-, and 12- Tube Test. There is also an option to use data from Wet Antecedent Conditions only, and options to define these conditions. Options for statistical parameters include choices of Alpha and Beta Level for Statistics.

There are also options to apply either the standard NSSP method to meet the NSSP criteria, in which the Geometric Mean must be less than 14 and the Estimated 90th percentile must be less than 49 for 3-tube Test, or a NSSP-CI method in which CI stands for Confidence Interval. The second choice is a more sensitive option in which the NSSP method is modified by using the upper limit of the 95% confidence interval.

Another option provided is to use censored data. If the value of MPN is reported with the less than sign (<), then it is a left-censored value. If the value of MPN is reported with the greater than sign(>), then it is a right-censored value. There are two methods to substitute these values: (1) The NSSP method, in which the program decreases or increases the value one significant figure. For example, a value less than 2 is substituted with 1.9 or (2) The ONE-HALF method, in which the program substitutes the value with one-half. For example a value less than 2 is substituted with 1. The default value is the NSSP option.

There are input screens for setting the parameters for the Old Closure Rule and the New Closure Rule, with 15 potential variables for each. There are six options available for both Primary Rules and Secondary Rules. There are also optional choices for Grace Periods, if they are included in the Rules. Once the parameters are set for the Old Rule, each, or all the parameters may be modified in the hypothetical new rule. When activated the program evaluates and calculates the

90^{th} percentile, and performs a simulation of rainfall events under application of the existing rule and the proposed new rule.

The first simulation result window exhibits a data sheet showing all the rainfall records generated, and the Site Status of the Region as either Open or Closed under the two rules. It also shows the Date and Time of the rainfall samples, the Cumulative 6-inch and 24-inch rainfall data, and the status of the Old Period and the New Period as being in the Grace Period, Waiting Period, Countdown Period (while the site is closed) and the Open Period. The data can also be filtered using three options to view the same parameters for the Site Status under the Old and New Rules showing records with different period codes, records with different status codes, and records with the same status codes. The data can also be exported to an Excel file for ancillary analysis.

The next simulation data sheet shows all the records of the fecal coliform samples taken as they apply to the Old and New Rules. The data presented shows the Region, Site, whether the site is closed under the Old status and the New status. It also exhibits the Date and Time of the sample, the fecal coliform Most Probable Number, the Sample database (type) and whether or not the sample was taken under Adverse Conditions. There are two additional filter options, by which the record of the real fecal coliform samples viewed represent records with different status Codes, or those with the same status Code.

The final output screen includes a 22-Section report, consisting of a summation of the statistical analyses that determines whether or not the hypothetical changes to the Old Rule can be considered for adopted as the New Rule for the shellfish harvest area (site). Sections 1 through Section 7 are descriptive statistics, in which the current rule and the proposed new rule are analyzed and contrasted. These sections provide information about sample numbers, Median, Percent MPN greater than 43, Geometric Mean, Log Average, Log Standard Deviation and Estimated 90^{th} Percentile for each rule when the site is open, and when the site is closed under the current rule, and open under the new rule. There are checkpoints that signal when the effort should be terminated if the Estimated 90^{th} percentile is greater than MPN 43, and the hypothetical changes would violate the NSSP.

Sections 8 through Section 11 are parametric statistics in which T-Test analyses compare (Current Closed - New Open against Current Open), (Current Open - New Closed against New Open) and (New Open against Current Open). The final statistical analyses determine whether the T-Tests show a significant difference at Alpha = 0.05; and interprets whether or not, the New Open is equal to the Current Open. If equal, this means that the data support the New Rule to be the same as the Current Rule, and the area may be considered for operation under the New Rule.

Sections 12 through 15 are nonparametric statistics, which are less accurate than parametric test and not normally applied. Nonparametric testing is used when the data samples are not thought to be normally distributed.

Sections 16 through 20 are informational sections that display depending on whether the results of the analysis give positive or negative results.

The final display screen has three levels of filters. Filter Level 0 is the default setting and displays all 15 sections of the report. Filter Level 1 displays five sections (2, 3, 4, 10, 11), which are important to the approval of a new rule. Sections 2, 3, and 4 are concerned with the NSSP criteria, and Sections 10 and 11 are the results of the parametric tests (T-Test). The T-Test compares the critical period with other periods. The Critical Period is defined as when fecal coliform samples were taken when the site is closed under the Old Rule, but open under the New Rule.

Filter Level 2 exhibits the same first three sections as described for Filter 1, except it also displays the non-parametric tests in Sections 14 and 15. The non-parametric, Wilcoxn Rank Sum Test also compares the critical period with other Periods, is employed when the data samples are not thought to be normally distributed, but is less accurate than the T-Test.

Filter Level 3 exhibits Sections 2, 3 and 4 and shows only the sections pertaining to NSSP regulations, while hiding the parametric and non-parametric sections.

Aquarius 2.0 also has two additional statistical packages for determining adequacy of sample sizes. The Sample Size program is built into the software and performs an analysis on appropriate statistical sections to determine if the sample size is adequate to perform a valid analysis, and exhibits the proper number of samples necessary to run the analysis. A second program, GPower, is a more sensitive and powerful sample size statistical program that by permission of its authors may be installed into Aquarius 2.0. It also possesses several analytical features that can be used in planning a sampling program and to determine the statistical power of the sampling protocol.

Specific instructions for software instillation and instructions for the use of ancillary programs, including GPower, Aquarium Tool, and the Rainfall Processing Program, which contains four modules Dup, Rev, Sum and Tip, are found in the appendices.

The manual is designed to take the user through five examples of the software's application, and increasing the user's knowledge base with each application. Of special value of the program is its ability to be used as an analytical tool in managing sanitation data, studying protocols in shellfish sanitation statistical sampling and as a training tool for agencies and the shellfish industry.

AQUARIUS v 2.0
Manual of Operations

Installation of Aquarius 2.0

Insert the Aquarius CD in the CD-ROM drive and follow on-screen prompts.

We recommend that everyone should be given Full control on the UCDAVIS folder, if the computer is used in 0n-admin mode. In addition, if you are not going to install the program on the C drive, then you must edit the cfg file and replace all C:\ with your drive of choice, for example E:\.

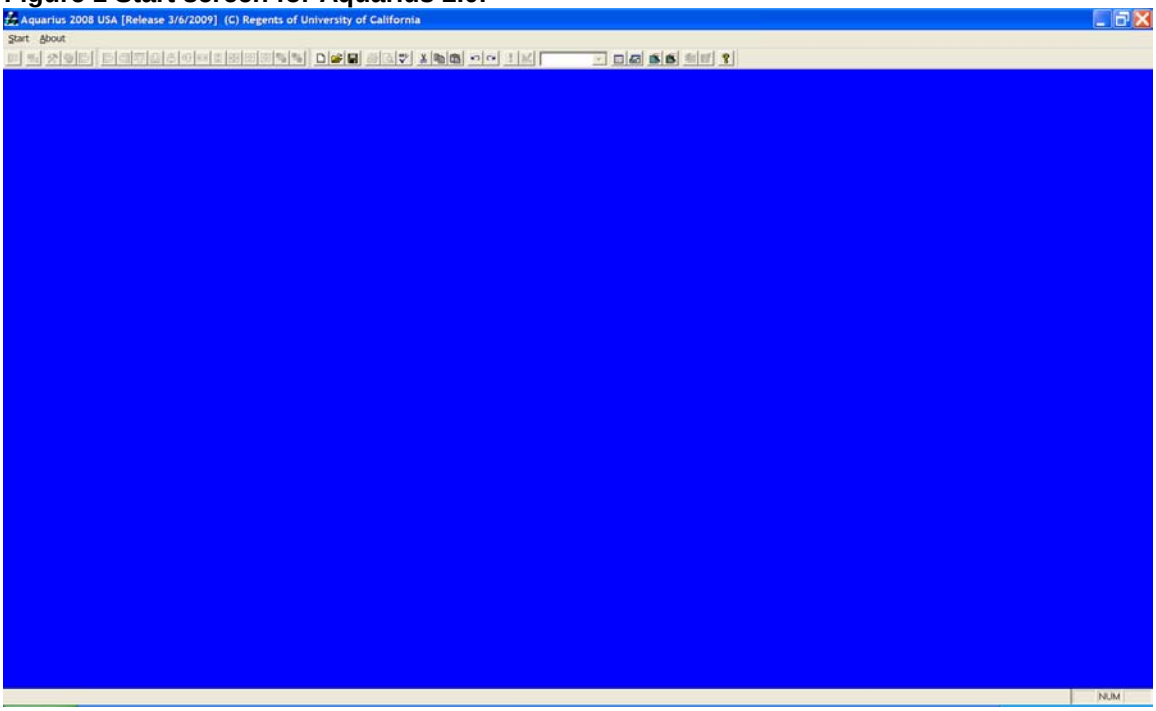
Please refer to Appendix A for specific instillation instructions.

Operation of Aquarius 2.0

During the installation of Aquarius 2.0, a Shortcut link will appear on your desktop

Double-click on the Launch Aquarius shortcut to activate the program. An opening screen will appear. (<u>If you wish to load new rainfall and fecal coliform databases, refer to Appendices A and B</u>).

Figure 1 Start screen for Aquarius 2.0.



Click on the Start button and a rainfall database will appear.

Figure 2. Rainfall database using the Eureka 01-2001 to 06-2003 example.

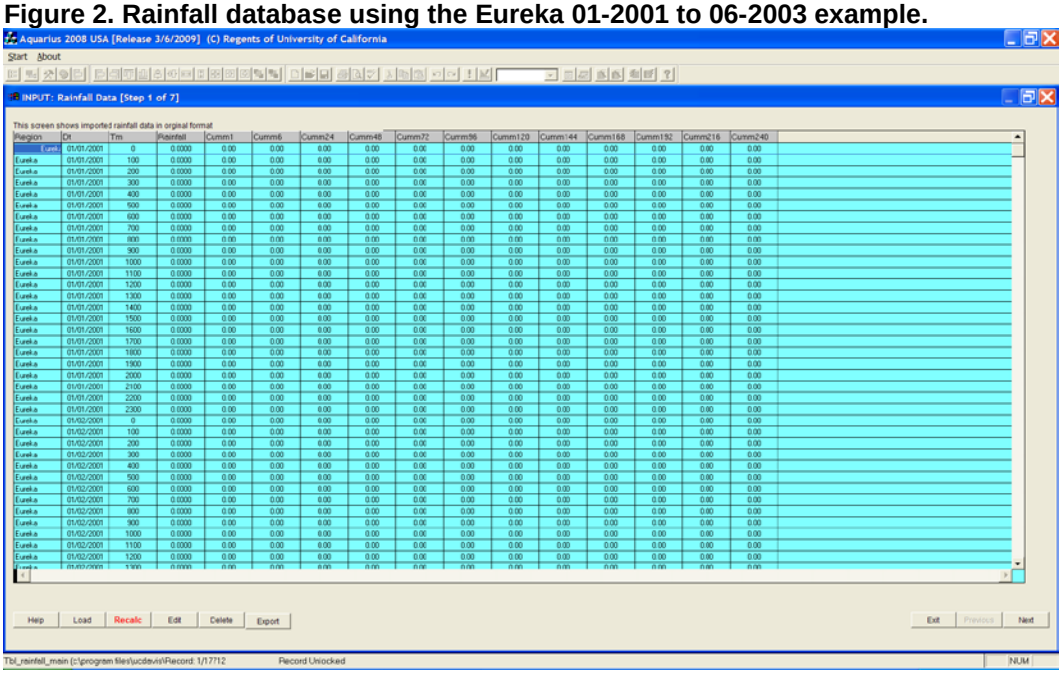
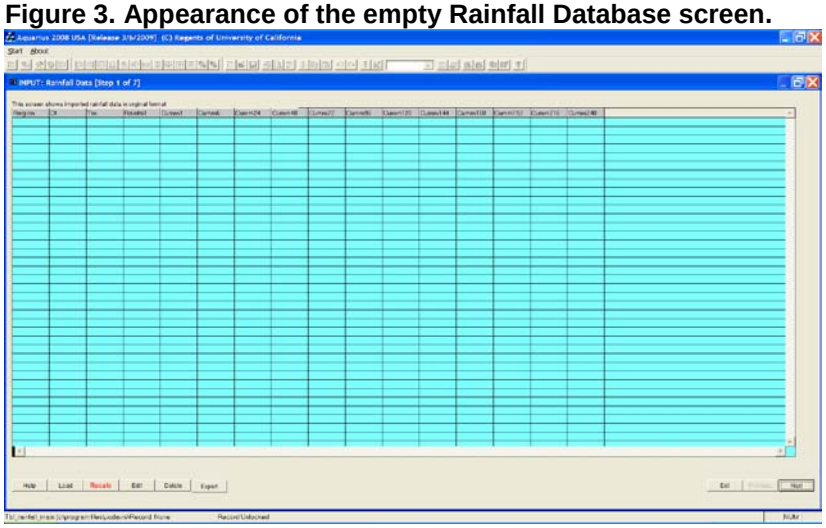


Figure 2 displays Input: Rainfall Data [Step 1 of 7]. Step 1 will display the last rainfall database that was loaded into the program. To demonstrate how to load a different database into the program, you can use the **Delete**, then the **Load** options.

Press the Delete Button on the menu at the bottom of the screen. You will be asked if you want to delete the currently exhibited rainfall database. **Press yes**.

The empty rainfall database screen will appear (Figure 3).

Figure 3. Appearance of the empty Rainfall Database screen.

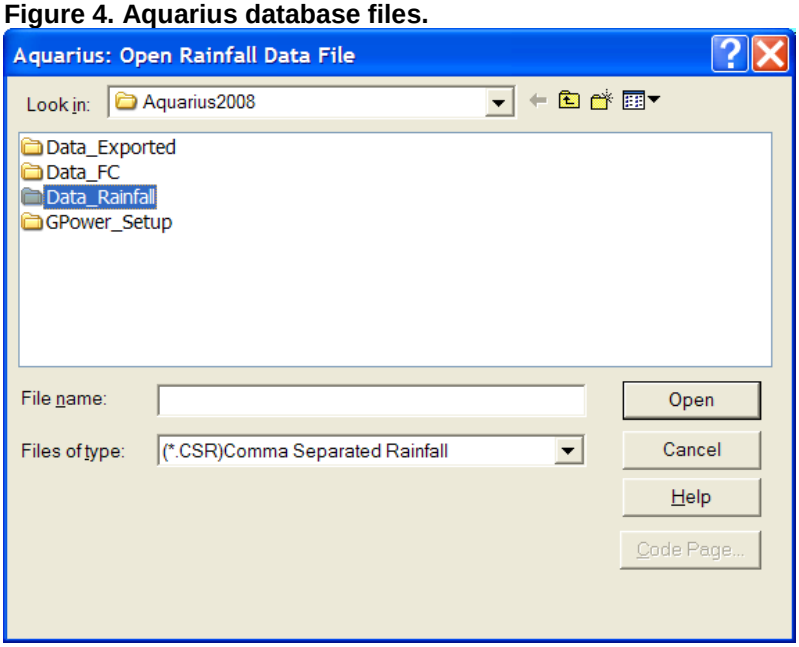


Loading Rainfall Database

When the empty Rainfall Database screen appears, **Press Load**

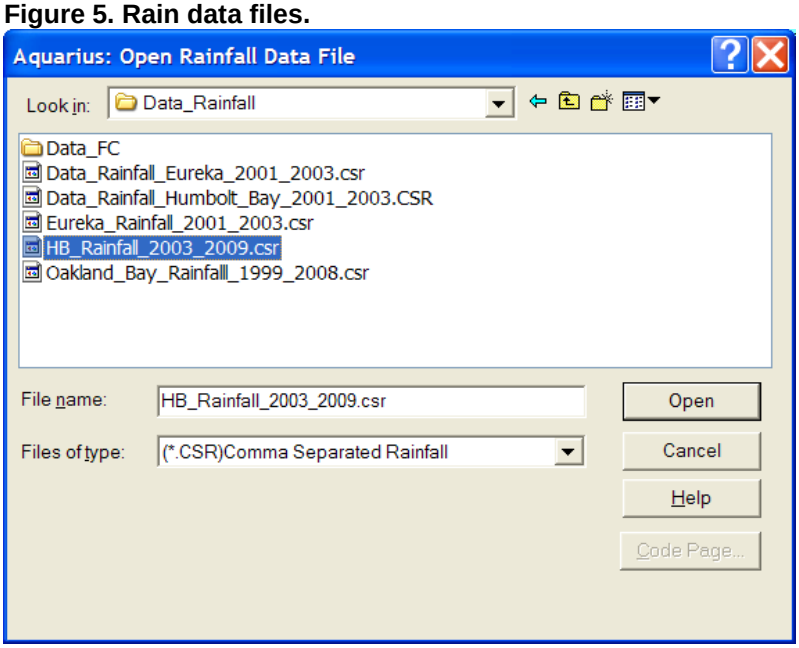
You will be asked, “Do you want to load rainfall data from a Comma Separated Rainfall file (*.CRS)?” **Press Yes**

Figure 4. Aquarius database files.



Click on Data_Rainfall to display rainfall files

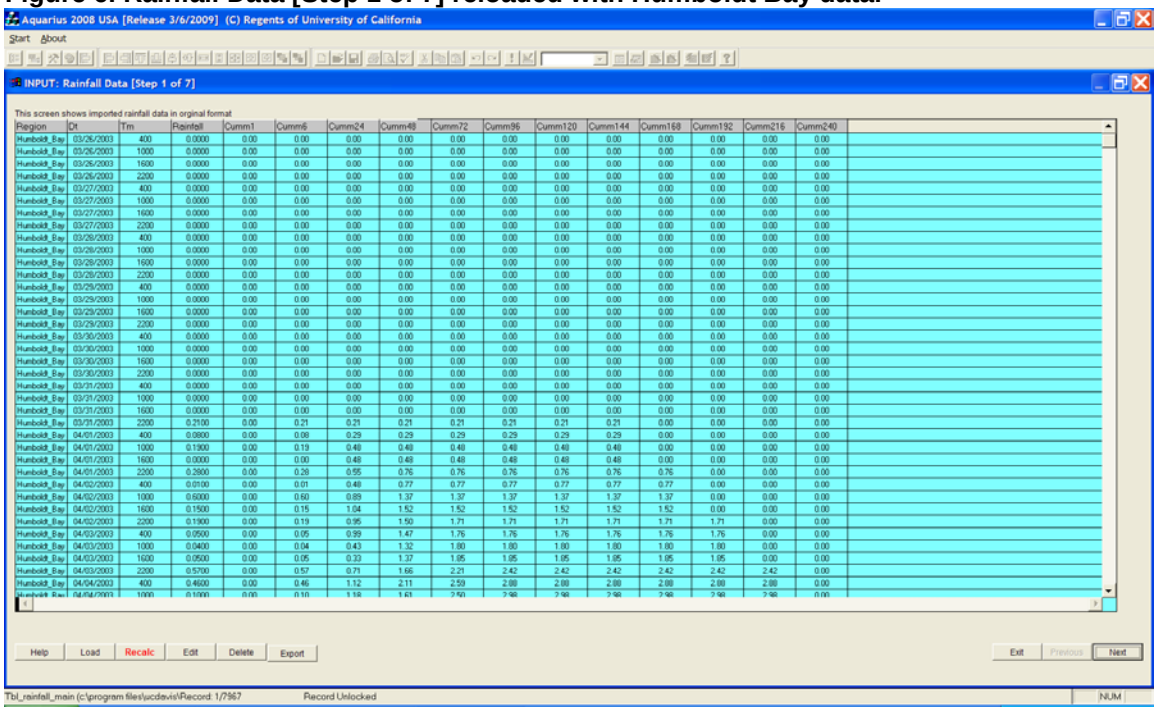
Figure 5. Rain data files.



Select HB_Rainfall_2003_2009.csr and **Press Open**.

The HB_Rainfall_2003_2009.csr file will be loaded into the Rainfall Database screen. This is the Humboldt Bay rainfall database for 2003 through 2009 in a comma separated rainfall (CSR) format.

Figure 6. Rainfall Data [Step 1 of 7] reloaded with Humboldt Bay data.



The next step is to recalculate the database. Aquarius 2.0 automatically recalculated the rainfall database whenever the new database is loaded.

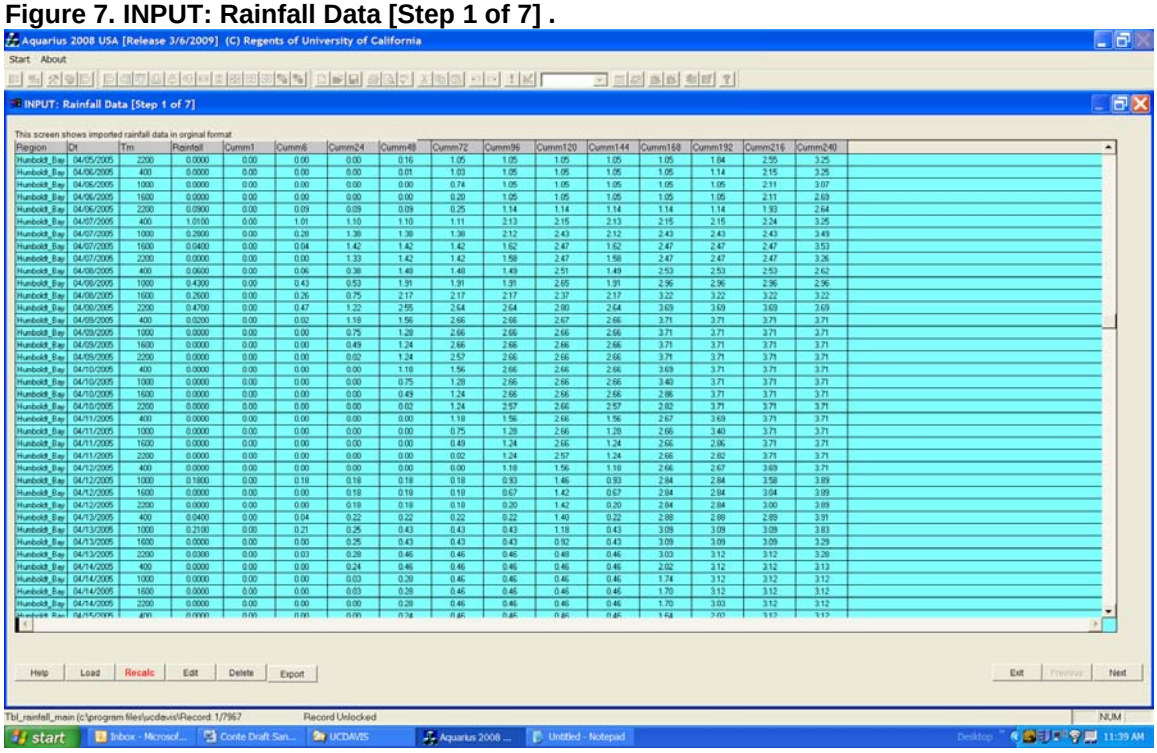
You can also press the Recalc button on the menu options at the bottom of the screen. You will be asked, “Do you want to recalculate cumulative rainfall values?”

Press Yes.

The rainfall database for Humboldt Bay will be recalculated as cumulative rainfall data from 24-hours to 240-hours and displayed as seen in Figure 7.

Rainfall Data: Rainfall data are recorded in inches, and must be collected at one, six, or 24 hours intervals with no missing values. Aquarius can import tipped rainfall data by converting it to hourly intervals. There are three utility programs for Aquarius called Dup, Sum and Tip. If loaded into Aquarius, these utility programs with their user’s manuals are in the **Data_wpp folder**. To run them you must navigate to this folder. The code wpp stands for Weather Processing Programs.

Figure 7. INPUT: Rainfall Data [Step 1 of 7] .



Rainfall Data Continued: The text file is a Comma Separated Rainfall (*.CSR) file, and is stored in the Aquarius' Data_Rainfall folder as Region, Date, Time and Rainfall.

```
Region Dt          Tm   Rainfall
Eurika,01/01/2005,0000,0.0045
Eurika,01/01/2005,0100,0.0045
Eurika,01/01/2005,0200,0.0045
Eurika,01/01/2005,0300,0.0045
Eurika,01/01/2005,0400,0.0045
Eurika,01/01/2005,0500,0.0045
Eurika,01/01/2005,2300,0.0045
Eurika,01/02/2005,0000,0.0045
```

The structure of the text file is as follows:

Region: Text,30 characters

Dt: Date, 10 characters (MM/DD/yyyy)

Tm: Numeric,4 digits (0000 to 2359 military time) or Text, 5 chars hh:mm (00:00 to 23:59 military time)

Rainfall: Numeric, 10 digits,4 decimals (99999.9999) (inch)

The best way to create this text file is to save your Excel spreadsheet as a Comma Separated Value (*.CSV) file and then change its extension to (*.CSR)

NAVIGATION: INPUT: Rainfall Data [Step 1 of 7]

[Help] Displays help information.

[Exit] Terminates the current Aquarius program.

[Next] Takes you to the next screen showing inputs for fecal coliform data.

[Recalc] This function recalculates the cumulative rainfalls from cumulative six hours to cumulative 240 hours (10 days cumulative).

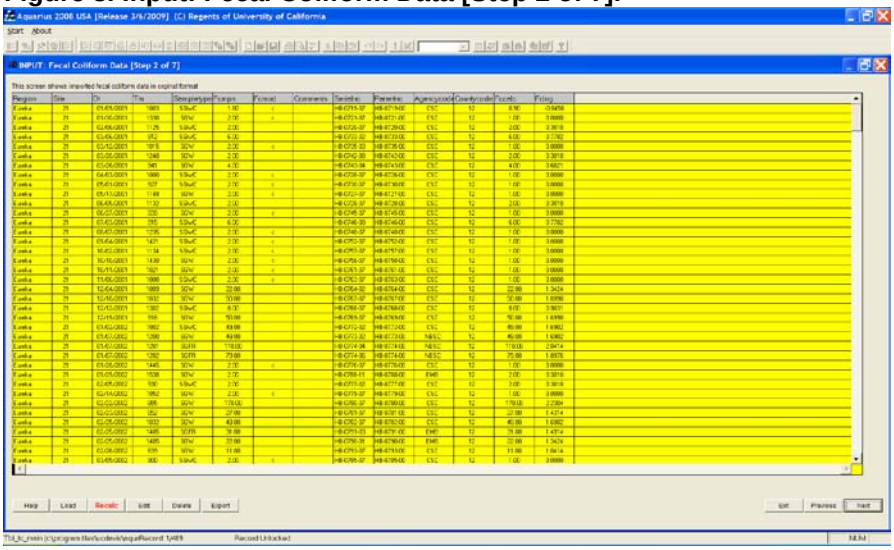
[Edit] This function allows you to edit the imported rainfall data in the Aquarius Table, but it does not modify the original source file (*.CSR) file. This allows the user to modify the data in “what if” scenarios, but does not alter the original record.

[Delete] This function allows you to delete the imported data from Aquarius. It does not delete the original source file (*.CSR) file.

[Export] This function allows you to export the rainfall data from Aquarius table onto an Excel file.

Press Next to access Input: Fecal Coliform Data [Step 2 of 7]

Figure 8. Input: Fecal Coliform Data [Step 2 of 7].

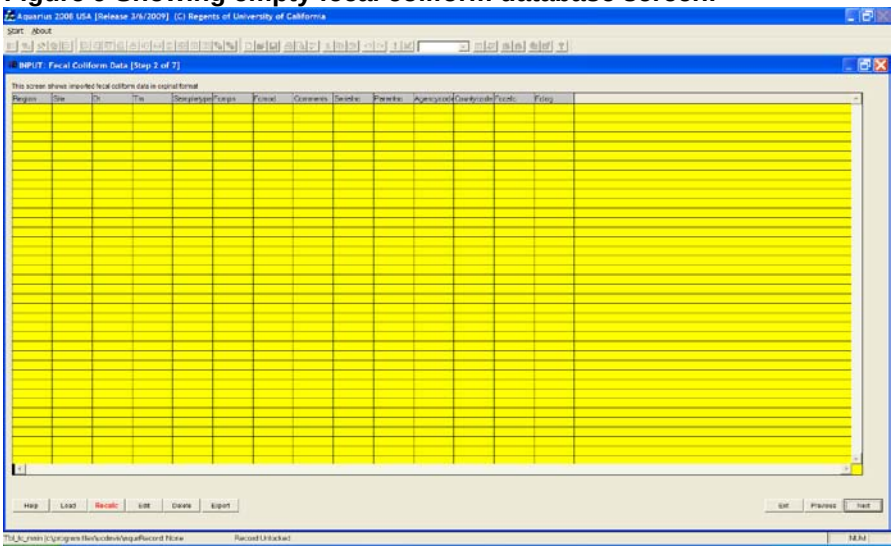


Step 2 of 7 will display the last fecal coliform database that was loaded into the program. To demonstrate how to load a different database into the program, you can use the **Delete**, then the **Load** options.

Press the Delete button on the menu at the bottom of the screen. You will be asked if you want to delete the currently exhibited fecal coliform database.

Press Yes, and the database will be deleted from the screen as seen in Figure 9.

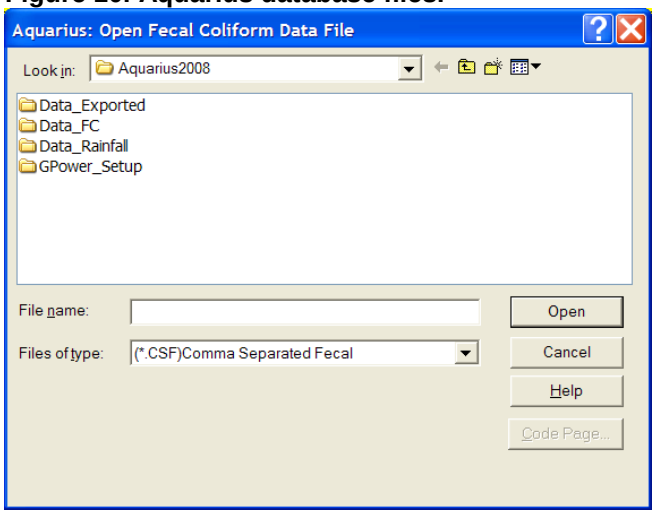
Figure 9 Showing empty fecal coliform database screen.



Loading Fecal Coliform Database

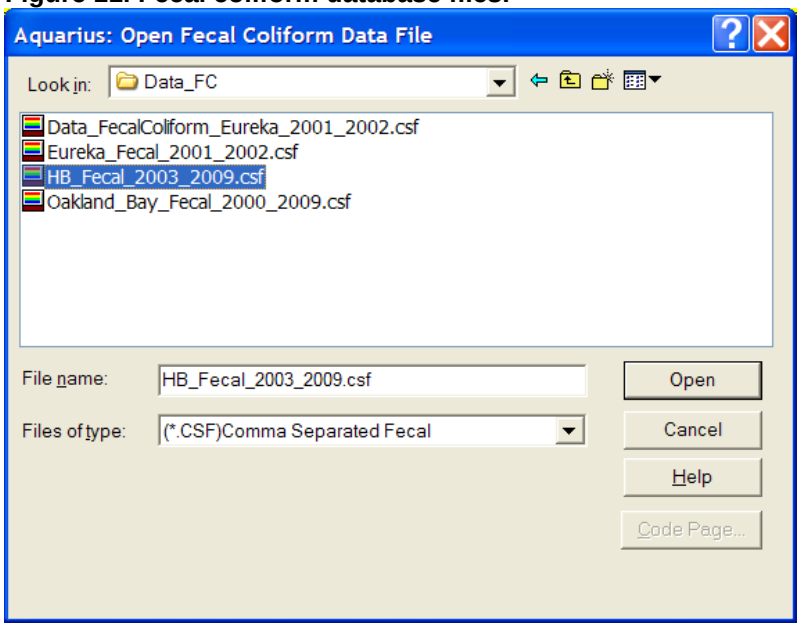
Press Load to access another Fecal Coliform database. You will be asked, “Do you want to load fecal coliform data from a Comma Separated Fecal Coliform file (*.CFS)?” **Press Yes**. The dialog box for Fecal Coliform files appears.

Figure 10. Aquarius database files.



Click on Data_FC to access fecal coliform files.

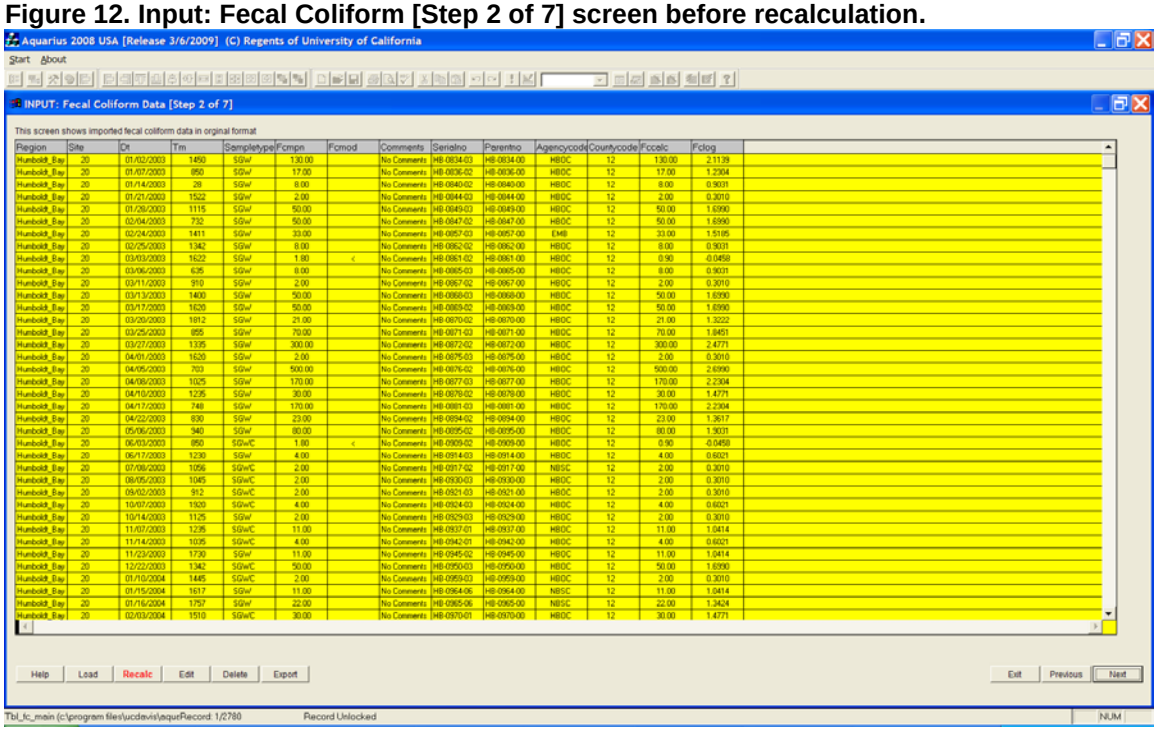
Figure 11. Fecal coliform database files.



Select HB_Fecal_2003_2009.csf and click **Open**.

Note: The Fecal Coliform data file must be a match to the Rainfall data file.

Figure 12. Input: Fecal Coliform [Step 2 of 7] screen before recalculation.



The last two columns (Fccalc and Fclog) appearing on the right of the database should now be recalculated. If no changes were made to the database, this would not be necessary; however, as a precaution, it should be done.

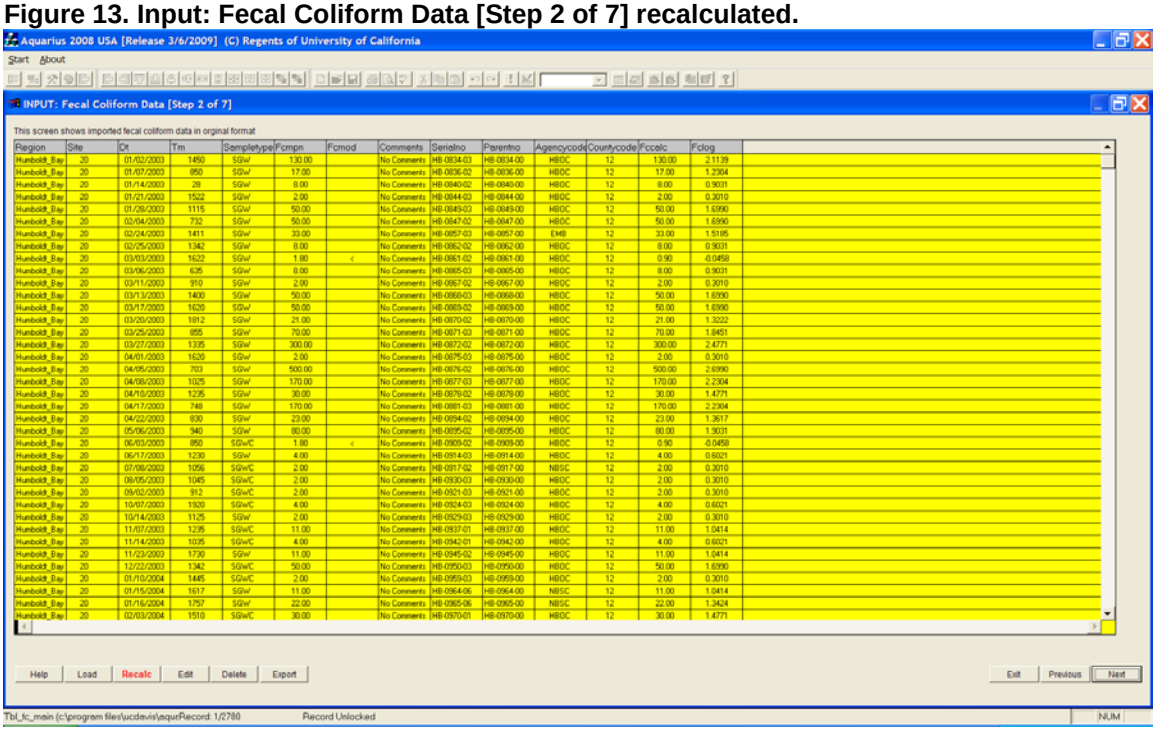
Press the **Recalc** button on the menu at the bottom of the database screen.

You will be asked, “Do you want to recalculate Fecal Coliform table?”

Press Yes.

The Fecal Coliform database for Humboldt Bay will be recalculated and displayed as seen in Figure 13.

Figure 13. Input: Fecal Coliform Data [Step 2 of 7] recalculated.



NAVIGATING Input: Fecal Coliform [Step 2 of 7]

[Help] Displays help information.

[Load] This function allows you to import FC data from a text file, update old records, or append new records to the FC table.

[Recalc] This function recalculates the log of the fecal coliform values.

[Edit] This function allows you to edit the imported data in the Aquarius table, but it does not modify the original source file (*.CSF)file.

[Delete] This function allows you to delete the imported data from Aquarius. It does not delete the original source (*.CSF) file.

[Export] This function allows you to export the fecal coliform data from Aquarius table onto an Excel file.

[Exit] Terminates the current Aquarius program.

[Previous] Takes you back to the Input Rainfall Data screen.

[Next] Takes you to the next screen for entering information for Regional inputs.

Fecal Coliform Database Files

The fecal coliform text file is a Comma Separated Fecal (*.CSF) file, and is stored in The Aquarius' Data_FC folder. The best way to create this file is to save your spreadsheet as a comma separated value (*.CSV) file and then rename its extension to (*.CSF).

Site: The Site column in the data file is used to build the pull-down menu for Site(s). You can use this column in your spreadsheet to combine or split your sites, before importing your data into Aquarius.

SampleType: This column is used to build the pull-down menu for the sample Type(s) field. You can use this column in your spreadsheet to mark your desired samples before importing your data into Aquarius.

The structure of the text file is as follows:

1. Region – The first column shows the region. The region in the Fecal Coliform data set must match the region in the rainfall data set. No space are allowed. Replace spaces with an underscore, for example, change Humboldt Bay to Humboldt_Bay. Do not enclose region name in double or single quotes. The maximum width of this column is 30 characters. Aquarius uses this column to build a drop down list for selecting region.

2. Site – The second column shows the sampling site. It can be number or text. Aquarius uses this column to build a drop down list for site.

3. Date – The third column shows date in the format of MM/DD/YYYY

4. Time – The fourth column shows the time in the military format hh:mm or in the numeric format. In the numeric format, the number 732 denotes 7:32am and the number 1411 denotes 2:11pm.

5. SampleType – The fifth column shows the sample type. It is text. Aquarius uses this column to build a drop down list for selecting sample type.

6. FCMPN – The sixth column shows the Fecal Coliform in MPN per 100 ml.

7. FCMOD – The seventh column shows the modifier for the fecal coliform value. This column either is blank or contains the less than sig (<) or the greater than sign (>).

8. Comments - The 8th column shows the Comments.

9. SerialNo – The 9th column shows the serial number. This can be used by state agency for tracking purposes.

10. ParentNo – The 10th column shows Parent No.

11. AgencyCode - The 11th column shows the Agency code. This is used by state agency for tracking purposes.

12. CountyCode – The last column shows the County Code.

It is important to arrange the 12 columns in the order described above. The first seven columns are used by Aquarius, and the last five columns (columns 8 through 12) are for State Agency tracking purposes.

The following listing is an example of Fecal coliform text file. Note that the heading must be is on a single line, but is broken here into two lines to fit the help file restrictions

Region,Site,Date,Time,SampleType,FcMPN,FcMod,Comments,SerialNo,
ParentNo,AgencyCode,CountyCode
Humboldt_Bay,25,1/2/2003,1425,SGW,50, ,,HB-0834-01,HB-0834-00,HBOC,12
Humboldt_Bay,26,1/2/2003,1435,SGW,70, ,,HB-0834-02,HB-0834-00,HBOC,12
Humboldt_Bay,20,1/2/2003,1450,SGW,130, ,,HB-0834-03,HB-0834-00,HBOC,12
Humboldt_Bay,27,1/2/2003,1506,SGW,500, ,,HB-0834-04,HB-0834-00,HBOC,12
Humboldt_Bay,27,1/7/2003,840,SGW,30, ,,HB-0836-01,HB-0836-00,HBOC,12
Humboldt_Bay,20,1/7/2003,850,SGW,17, ,,HB-0836-02,HB-0836-00,HBOC,12
Humboldt_Bay,53,1/7/2003,900,SGWC,27, ,,HB-0828-01,HB-0828-00,CSC,12
Humboldt_Bay,26,1/7/2003,904,SGW,11, ,,HB-0836-03,HB-0836-00,HBOC,12
Humboldt_Bay,52,1/7/2003,906,SGWC,17, ,,HB-0828-02,HB-0828-00,CSC,12
Humboldt_Bay,25,1/7/2003,908,SGW,11, ,,HB-0836-04,HB-0836-00,HBOC,12
Humboldt_Bay,51,1/7/2003,911,SGWC,23, ,,HB-0828-03,HB-0828-00,CSC,12
Humboldt_Bay,41,1/7/2003,920,SGWC,17, ,,HB-0828-04,HB-0828-00,CSC,12
Humboldt_Bay,45,1/7/2003,925,SGWC,13, ,,HB-0828-05,HB-0828-00,CSC,12
Humboldt_Bay,22,1/7/2003,930,SGWC,11, ,,HB-0828-06,HB-0828-00,CSC,12
Humboldt_Bay,21,1/7/2003,937,SGWC,13, ,,HB-0828-07,HB-0828-00,CSC,12
Humboldt_Bay,24,1/7/2003,1007,SGWC,8, ,,HB-0828-08,HB-0828-00,CSC,12

The previous descriptions provide an introduction to the databases by which Aquarius v.2 operates. To illustrate the operation of the program we will present five basic examples of the programs applications.

Example 1 An example in which the hypothetical new rule is relaxed and opens up more days for harvest, and one that provides an example of an outcome in which the statistical analysis supports the hypothetical new rule.

Example 2 An example in which the hypothetical new rule is relaxed and opens up more days for harvest, and one that provides an example of an outcome in which the statistical analysis does not support the hypothetical new rule.

Example 3 An example in which the hypothetical new rule is more restrictive and closes more days for harvest, and one that provides an example of an outcome in which the statistical analysis supports the hypothetical new rule.

Example 4 An example in which the hypothetical new rule is more restrictive and closes more days for harvest, and one that provides an example of an outcome in which the statistical analysis does not support the hypothetical new rule.

Example 5 An example in which a group of harvest sites are compared with a second group of harvest sites to determine if there are significant differences among the sites.

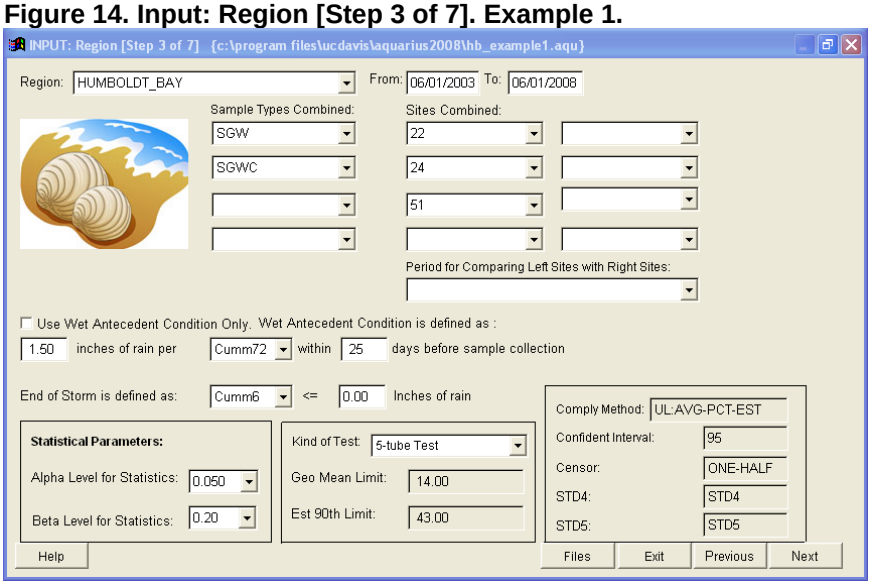
While the program is running and using the current databases for Humboldt_Bay rainfall and fecal coliform, **Press Next** and set up the scenario for Example 1.

Go to next page.

Example 1. In example one, we will set up a scenario in which a hypothetical new rule opens up more days for harvest, and one that provides an example of an outcome in which the statistical analysis supports the hypothetical new rule. This will allow us to demonstrate fundamental features of the program, and demonstrate how the analytical results screen displays these positive results.

[Next] takes you to Figure 14, the next screen for entering information for Regional inputs.

Figure 14. Input: Region [Step 3 of 7]. Example 1.



Note: The title line of the dialog box reads:

INPUT: Region [Step 3 of 7] {c:\program files\ucdavis\aquarius2008\hb_Example1.aqu}

<u>The Aquarius program automatically reloads the data variables used in the last analytical run. These variables may be changed to run a new analysis</u>.

Parameters for Example 1 Analysis: First Input Screen Figure 14

Region = Humboldt_Bay; **From** = 06-01-03 to 06-01-2008
Sample Types Combined = SGW and SGWC;
Sites Combined = 22; 24; 51; Wet Antecedence **not checked**;
Statistical Parameters Alpha = 0.050; Beta = 0.20;
Kind of test = 5-tube test (Geo Mean Limit = 14; Est 90th Limit = 43.0)
Comply Method = UL:AVG_PCT-EST
Confident Interval: 95
Censor: ONE-HALF
STD4: STD4
STD5: STD5

The Input Region screen allows input of variable data options to the selected Shellfish growing region.

Region: Allows you to select the specific shellfish growing region to be analyzed for potential rule change.

From: Allows you to select the specific time range from which the rainfall and fecal coliform data will be extracted from the data sets for the analysis.

Sample Types Combined: Allows for the selection of up to 4 separate fecal coliform sample types to be included in the analysis.

Sites Combined: Allows for the combination of up to 8 sites to be included in the analysis.

Period for Comparing Left Sites with Right Sites: If you select a value for this field, then Aquarius compares the sites in the left column with sites in the right column. The sites in the left column will be combined and the sites in the right column will be combined and then these two groups will be compared with each other using parametric t-test and non-parametric T-test. The results will be posted in the sections 16 to 20 of the output report. If you leave this field blank, Aquarius will skip site comparison.

Wet Antecedent Condition Only: Provides an option for the use of adverse condition data in the analysis. If wet antecedent data is checked, parameters defining the conditions are defined by the options in the boxes provided and Aquarius uses only those samples.

Setting the Level of Statistical Analysis: Information - Applying the null hypothesis to Aquarius: When the 'null hypothesis' is applied to Aquarius, it infers that that there is no real difference between the true value of fecal coliform under the existing closure rule and the new proposed closure rule. It implies that the conditions of the new proposed rule is same as the old rule, and could be approved by the regulatory agency.

Alpha Level for Statistics: Allows three options of statistical analysis at the Alpha level. The alpha level is usually set to 0.05, implying that you are willing to accept 5% chance of rejecting the null hypothesis when it should not be rejected.

Beta Level for Statistics: Allows three options of statistical analysis at the Beta level. The beta level is usually set to 0.20, implying that you are willing to accept a 20% chance for error in accepting the null hypothesis when it should not be accepted.

Kind of Test: The program allows seven options for conditionally approved growing areas: 3-tube test, 5-tube test, 12-tub test, Membrane Filter (MF) test, Restricted 3-tube test, Restricted 5-tube test and Restricted 12-tube test.

Each option displays the corresponding Geometric Mean limit and Estimated 90th Percentile Limit. The limits are specified in the Aquarius Configuration file. The limits are expressed as Most Probable Number per 100 ml

Compliance Method: This displays the method is used to check if the descriptive statistics meet the NSSP fecal coliform water quality standard. For more details see the Aquarius Configuration section at the end of this help message.

Censor: This displays the method is used to substitute values when the MPN is less than or greater than a certain limit. For more details see the Aquarius Configuration section that follows.

STD4: Denotes the Standard Deviation of the Critical Period (Old Closed, New Open). For more details see the Aquarius Configuration section that follows.

STD5: Denotes the Standard Deviation of the Critical Period (Old Open, New Closed). For more details see the Aquarius Configuration section that follows.

Navigating Input: Region [Step 3 of 7]

[Help] Displays help messages

[Files] Provides an option to Open an Aqua File that was previously saved, Save a File, Save as an Aqua File, or Reload a Configuration File.

[Exit] Exits the program

[Previous] Takes you back to the previous screen

[Next] Takes you to the next screen

Aquarius Configuration File: The configuration file for Aquarius is a text file called Aquarius.cfg. This file is resides in the Aquarius main folder and can be edited by the Notepad program. Make sure you do not change the labels at the left of the equal signs. You can change the values at the right of equal signs. If you delete this file, Aquarius will generate a default file as a replacement. This file has 22 lines as follows:

Line 1 - Compliance Method

If you are using Systematic Random Sampling, set the Comply Method to AVG-PCT-EST

The AVG code denotes the [Geometric Mean]. It says that the [Geometric Mean] must be less than 14

The PCT code denotes the [Percentage Factor]. It says that there shall not be more than 10 percent samples greater than the limit.

The EST code denotes the [Est 90th Percentile]. It says that the [Est 90th Percentile] must be less than 43 for 5-tube Test.

If you are not using Systematic Random Sampling, set the Comply Method to AVG-PCT

To incorporate the effect of sample size, a more conservative method is to calculate the Upper limit of the [Geometric Mean] and the [95% confident interval]. To use this conservative method, insert the UL Code to the beginning of the Comply Method:

Valid values are:

AVG-PCT-EST
AVG-PCT
UL:AVG-PCT-EST
UL:AVG-PCT

The default value is UL:AVG-PCT-EST

Notes on Compliance methods:

1. Aquarius calculates five parameters:

i. AVG – Geometric Mean
ii. PCT – Percentage Factor
iii. EST - Estimated 90th Percentile
iv. UL:AVG – Upper Limit of Geometric Mean
v. UL:EST – Upper Limit of Estimated 90th Percentile

2. The user can specify any combination of these five parameters in the configuration file for the Compliance Method. The default is (f), but can be changed by the user.

a) AVG-PCT - Uses Geometric Mean and Percentage Factor for compliance

b) AVG-EST - Uses Geometric Mean and Est. 90th Percentile for compliance.

c) AVG-PCT- EST - Uses Geometric Mean, Percentage Factor and Est. 90th Percentile for compliance.

d) UL: AVG-PCT - Uses Upper Limit of Geometric Mean and Percentage Factor for compliance

e) UL: AVG-EST - Uses Upper Limit of Geometric Mean and Upper Limit of Est. 90th Percentile for compliance.

f) UL: AVG-PCT- EST - Uses Upper Limit of Geometric Mean, Percentage Factor and Upper Limit of Est. 90th Percentile for compliance.

3. The configuration file, Aquarius.cfg, is has the ability to specify the confidence level (80 or 90 or 95 percent). The Confidence Interval is used to calculate upper limit for mean and standard deviation.

Confidence Interval of Mean

For 80% confidence interval the Z(alpha/2) = 1.283
For 90% confidence interval the Z(alpha/2) = 1.645
For 95% confidence interval the Z(alpha/2) = 1.96

Confidence Interval of Variance

For 80% confidence interval the p for Chi-Square is 0.90
For 90% confidence interval the p for Chi-Square is 0.95
For 95% confidence interval the p for Chi-Square is 0.975

4. In the Aquarian Tool, the user can set the confidence interval from 80 to 90 or to 95 percent.

5. The ability to edit the configuration file, Aquarius.cfg, is added to the Files dialog box.

6. The Load button in rainfall data and fecal coliform data, now automatically calculates the values after loading the data, although the fecal coliform must be recalculated, if you change the censor method.

Line 2 - Censor Method

If the value of MPN is reported with the less than sign (<) then it is a left-censored value. If the value of MPN is reported with the greater than sign (>) then it is a left-censored value. There are two methods to substitute these values.

NSSP method: Decrease or increase the value one significant figure. For example a value less than 2 is substituted with 1.9. (2) ONE-HALF method – Substitute the value with one-half. For example a value less than 2 is substituted with 1. **The default value is ONE-HALF.**

Valid values for Censor Method:

NSSP
ONE-HALF

Line 3 – STD4: Standard Deviation for Critical Period [Old Close, New Open). The critical period usually has fewer samples than other periods. To have a more conservative approach, you can use the standard deviation of close periods rather than its own standard deviation. To have a more liberal approach, you can use the standard deviation of open periods rather than its own standard deviation. You can specify your choice in the configuration file in the section ladled STD4. STD stands for Standard Deviation and 4 denotes the section 4 in the printout, that is, the critical period (Old Close, New Open). The default value is STD4

Valid Values for STD4:

STD4 – Standard deviation of critical period [Old Close, New Open)
Section 4 in printout
STD2 – Standard deviation of (Old Open) Section 2 in printout
STD3 – Standard deviation of (New Open) Section 3 in printout
STD6 – Standard deviation of (Old Close) Section 7 in printout
STD7 – Standard deviation of (New Close) Section 8 in printout

Line 4 – STD5: Standard Deviation for Critical Period [Old Open, New Close) The critical period usually has fewer samples than other periods and to have a more conservative approach, you can use the standard deviation of close periods rather than its own standard deviation. To have a more liberal approach, you can use the standard deviation of open periods rather than its own standard deviation. You can specify your choice in the configuration file in the section labeled STD5. STD stands for Standard Deviation and 5 denotes the section 5 in the printout, that is, the critical period (Old Open, New Close). The default value is STD4

Valid Values for STD5:

STD5 – Standard deviation of critical period [Old Open, New Close)
Section 5 in printout
STD2 – Standard deviation of (Old Open) Section 2 in printout
STD3 – Standard deviation of (New Open) Section 3 in printout
STD6 – Standard deviation of (Old Close) Section 7 in printout
STD7 – Standard deviation of (New Close) Section 8 in printout

Line 5 - GeoMean Limit for 3-tub test
The NSSP limit is 14.0. Do not change it

Line 6 - GeoMean Limit for 5-tub test
The NSSP limit is 14.0. Do not change it

Line 7 - GeoMean Limit for 12-tub test
The NSSP limit is 14.0. Do not change it

Line 8 - GeoMean Limit for MF test
The NSSP limit is 14.0. Do not change it

Line 9 - Est90th Limit for 3-tub test
The NSSP limit is 49.0. Do not change it

Line 10 - Est90th Limit for 5-tub test
The NSSP limit is 43.0. Do not change it

Line 11 -Est90th Limit for 12-tub test
The NSSP limit is 28.0. Do not change it

Line 12 -Est90th Limit for MF test
MF denotes Membrane Filter test. The NSSP limit is 31.0. Do not change it

Line 13 – Adverse GeoMean Limit for 3-tub test
The NSSP limit is 88.0. Do not change it

Line 14 - Adverse GeoMean Limit for 5-tub test
The NSSP limit is 88.0. Do not change it

Line 15 - Adverse GeoMean Limit for 12-tub test
The NSSP limit is 88.0. Do not change it

Line 16 – Adverse GeoMean Limit for MF test
The NSSP limit is 88.0. Do not change it

Line 17 - Adverse Est90th Limit for 3-tub test
The NSSP limit is 300.0. Do not change it

Line 16 - Adverse Est90th Limit for 5-tub test
The NSSP limit is 260.0. Do not change it

Line 19 - Adverse Est90th Limit for 12-tub test
The NSSP limit is 173.0. Do not change it

Line 20 - Adverse Est90th Limit for MF test

MF denotes Membrane Filter test. NSSP has not reported a limit for this category, therefore this is just a place holder for future use.

Line 21 – GPower: The GPowerNT.exe is a program to calculate sample size. By default it is installed on the C drive in its default path. If you have installed it in different path, specify the new path in the configuration file in the section labeled GPower. The default value is C:\Program Files\GPower 3.0\GPowerNT.exe

Line 22 – Browser: The path to Microsoft Web Browser, ieexplore.exe. Aquarius uses this program to browse the web. The default path is: C:\Program Files\Internet Explorer\iexplore.exe

Line 23 - URL ShellfishGuide: The web address for [National Shellfish Sanitation Program, Guide for the Control of Molluscan Shellfish]. The current web address is: http://www.cfsan.fda.gov/~ear/nss4-toc.html

Line 24 – URL AquariusWebsite: The web address for Aquarius web site. The current address is:

http://animalscience.ucdavis.edu/extension/Software/Aquarius/

Press Next to go to the next screen, Figure 15.

Figure 15. INPUT: Closure Rules [Step 4 of 7]. Example 1.

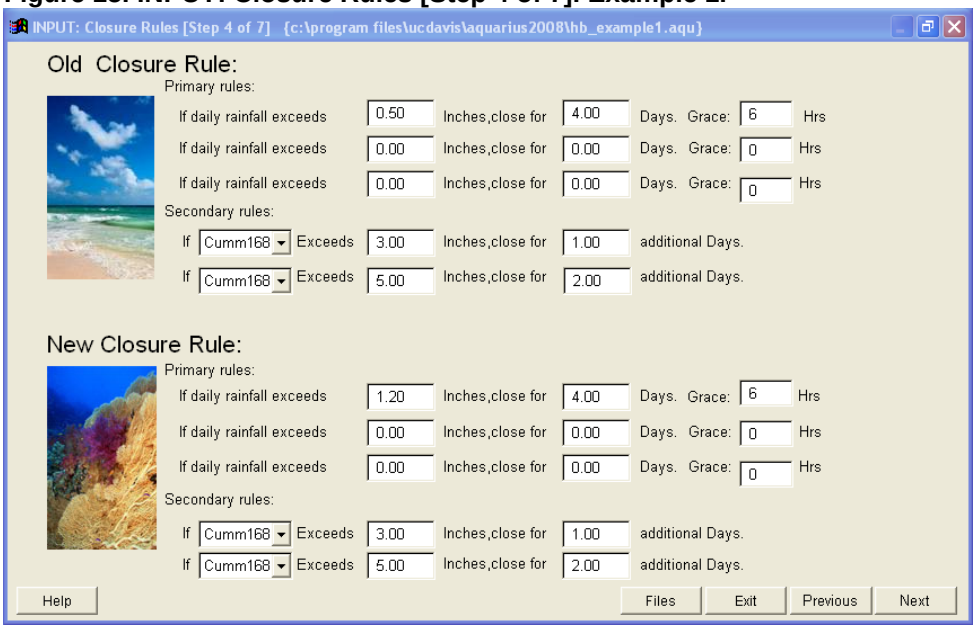


Figure 15, Screen Step 4 of 7 is an input screen designed to define the existing Old Rule, which appears at the top of the screen, and the hypothetical New Rule, which appears at the bottom. Most states, or provinces in other countries, have relatively simple closure rules for rainfall associated shellfish harvest closures, many with only one or two variables. California is among those states with complex rules. The Aquarius program allows up to fifteen variables in a single rule. The simulation engine and statistical packages within the program are robust and capable of handling the complexities of such an analysis.

Old Closure Rule: The input data for the Old Closure Rule allows up to 15 variables describing, and includes the Primary rules (9 variables) and Secondary rules (6 variables).

Primary rule: The Primary Rule (9 options) of the Old Rule provides three options if the daily rainfall exceeds a given amount of inches, and three options for the number of days closed. There are also three options for Grace periods.

Grace Period: The grace period, for example, may be the amount of time following a rainfall event and before the watershed delivers fecal coliform to the growing area. This is usually based on results of previous data analysis.

There are also six options for Secondary Rules based on cumulative rainfall, the amount of rainfall and the number of additional days that the area is closed.

New Closure Rule: The hypothetical, New Closure Rule has the identical 15 options as the Old Rule. It allows the user to provide the parameters describing the Primary rules and Secondary rules associated with the New Closure Rule.

Navigating Screen Step 4 of 7

[Help] Displays help message.

[Files] Displays a dialog box with five options: Open Aqua File (Allows you to open a previous saved file containing a set of input options); Save Aqua File (Allows you to save a set of input options in a file; Save As Aqua Files (Allows you to save a set of input options as a specific file; Edit Configuration File (Allows you to edit the configuration file to a specific set of NSSP criteria); and Reload Configuration File (Allows you to reload a saved set of criteria).

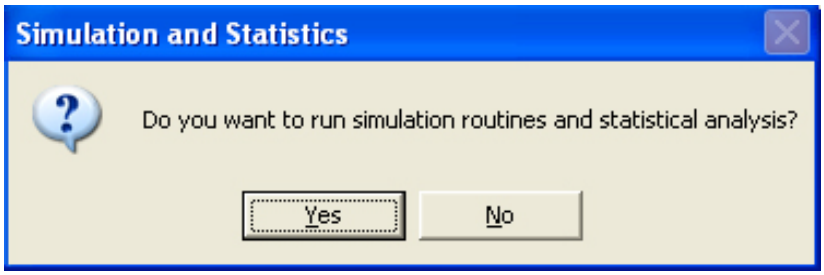
After editing a configuration file, you should save it with a specific name, and reload the file for the changes to take effect.

[Exit] Terminates Aquarius program.

[Previous] Takes you back to the previous screen showing the input table for fecal coliform data.

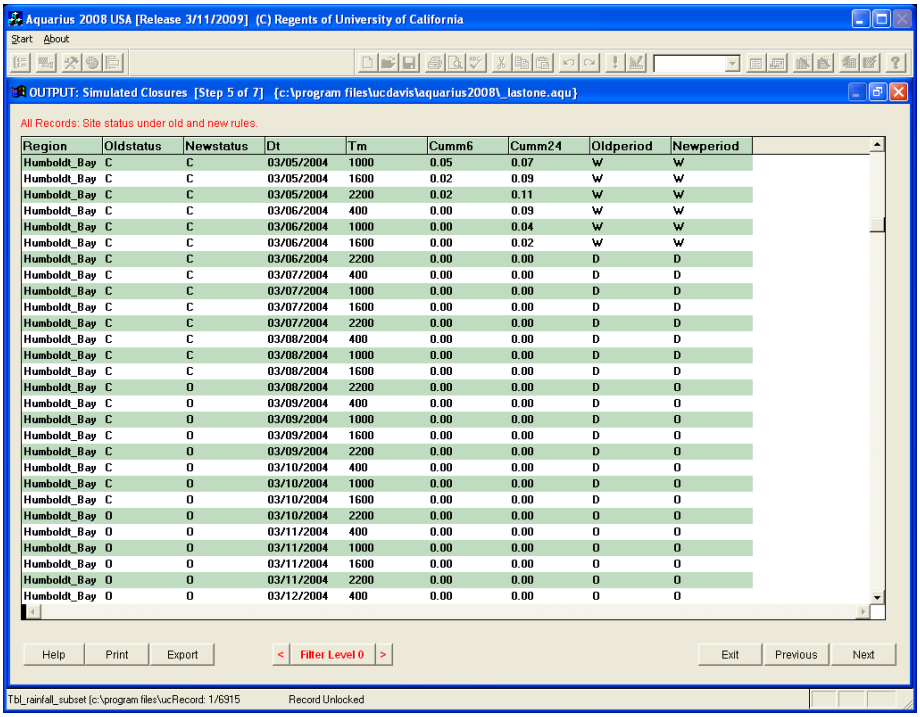
[Next] Registers the options entered for the old closure rule and the new proposed rainfall closure rule, and asks if you want to run the simulation routines and statistical analysis.

Press Next to run the simulation and statistical analysis. You will be asked, “Do you want to run the simulation and statistical analysis?”



Press Yes. You will receive the message, “Evaluating Closure Rules … Wait …” Following the simulation and analysis the first of three data result windows will appear as displayed in Figure 16.

Figure 16. OUTPUTS: Simulated Closures [Step 5 of 7]. All records and site status under the Old and New Rule. Example 1. Viewed as Filter Level 0.



The screen SIMULATION OUTPUT: Simulated Closures [Step 5 of 7] shows all records and site status under the Old and New Rules. Displays the simulated

closure periods using only Real Rainfall Data applied to the Old Rules and the hypothetical New Rules. The screen has been moved vertically about 1/5 the distance of the entire sheet to display more of the different codes.

The table shows all data for the region Humboldt_Bay, the Site status as either Open or Closed under the Oldstatus and Newstatus, Date, Time, Cumulative 6-hour and Cumulative 24-hour rainfall, and the sequence (algorithm) status of the Oldperiod and the hypothetical Newperiod.

Codes for OldStatus and NewStatus

O: Open Status - The shellfish growing site is open.

C: Closed Status - The shellfish growing site is closed.

Codes for Oldperiod and Newperiod

G: Grace period - The rainfall exceeds the threshold and the site is in the Grace period. The site is still open during this period.

W: Waiting period - The Grace hours are used up and the site is in the Waiting period, that is it is waiting for the end of storm event. The site is closed during this period.

D: Count Down Period - The End of Storm event has happened and the site is in the Count Down period, that is it is counting down the Hours Closed. The site is closed during this period.

O: Open Period - The Hours Closed counter is used up, and the site is Open.

Filter Levels: Allows filtration of data to show specific aspects of the analysis

Filter Level 0: Default Screen – All records, and showing Open and Closed status of site under Old and New Rules.

Filter Level 1: Shows the sites under the Old and New Rules, when the sites are open under both rules.

Filter Level 2: Shows the sites under the Old and New Rules, when the sites are closed under both rules.

Filter Level 3: Shows the sites under the Old and New Rules, when the sites are closed under the old rule, but open under the hypothetical new rule.

Algorithm: In the Help menu, this button opens a diagram that describes how the sequence of events occur in the Aquarius program (Press algorithm button in the help menu)

Navigating SIMULATION OUTPUT: Simulated Closures [Step 5 of 7]

[Help] Displays help message.

[Print] Prints the data sheet.

< [Filter Level] > Allows filtration of data to show specific aspects of the analysis.
[Export] Allows you to export files to an Excel spreadsheet.

[Exit] Terminates Aquarius program.

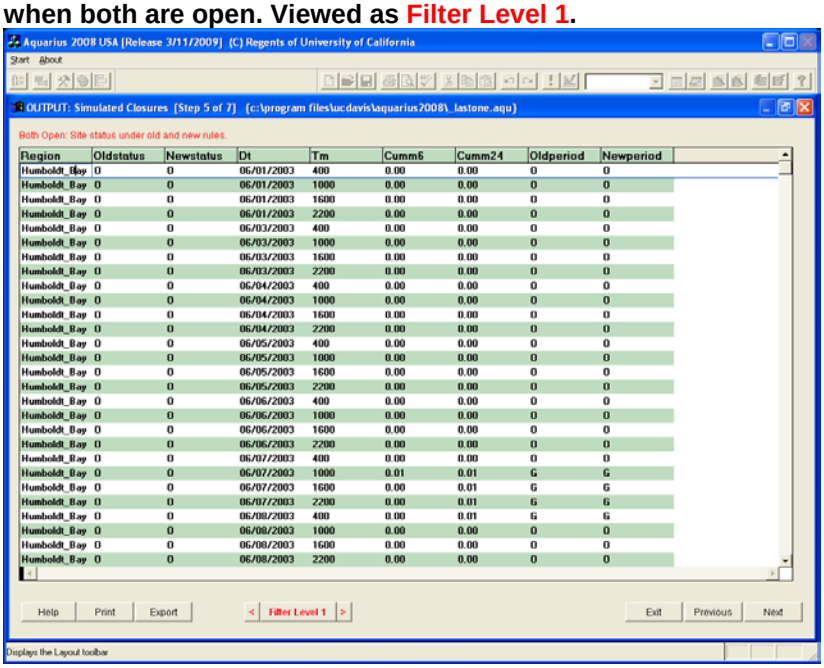
[Previous] Takes you back to the previous screen showing the input tables for the Old Rules and hypothetical New Rules.

[Next] Shows All Records: Real Fecal Coliform samples under old and new rule.

Now, while you are still in Step 5 of 7, **set the Filter level to Level 1.**

Filter Level 1 shows the sites being examined under the Old and New Rules, when the site is open under both rules (Figure 17).

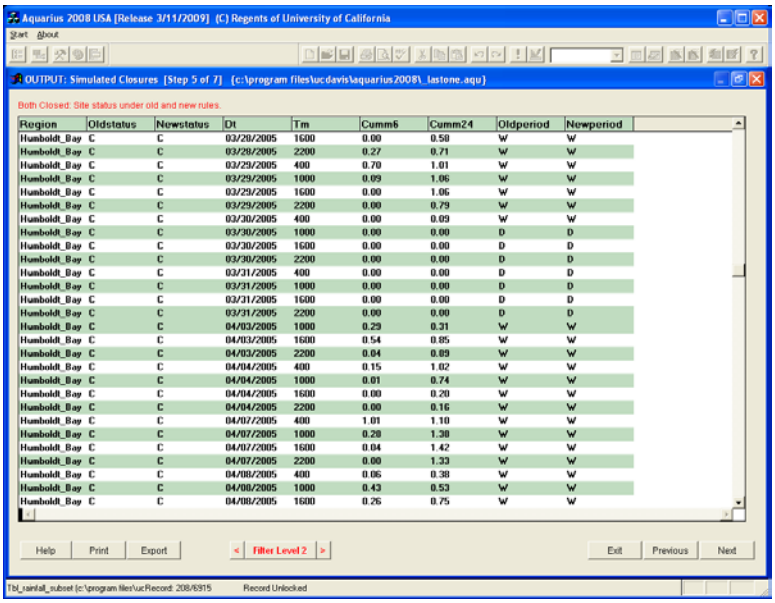
Figure 17. [Step 5 of 7]. Site status under Old and New Rule when both are open. Viewed as Filter Level 1.



Note that the sites are open under both the old rule and the new rule (2nd and 3rd columns), and either open, or open under the grace period under both rules (8th and 9th columns).

While you are still in Step 5 of 7, **set the Filter level to Level 2.** Filter Level 2 Shows the site under the Old and New Rules, when the site is closed under both rules (Figure 18).

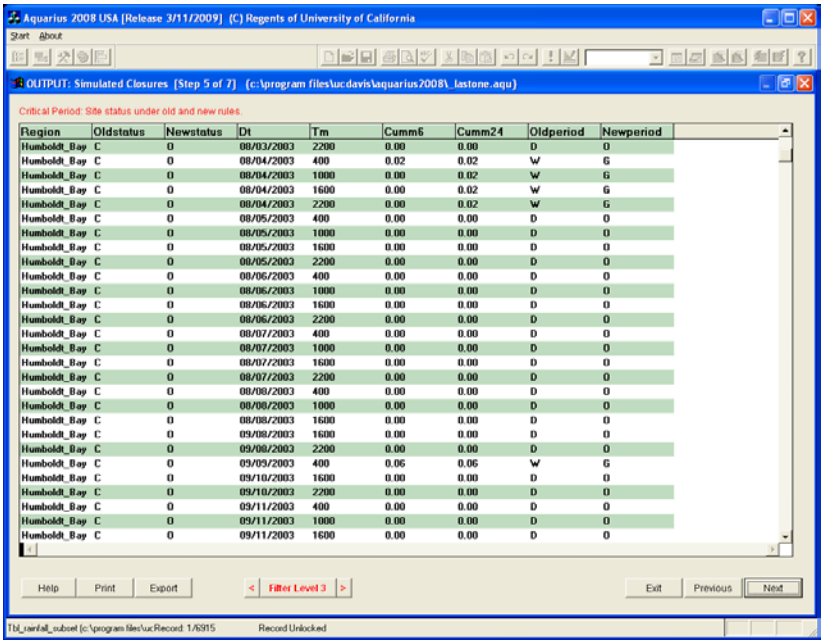
Figure 18. [Step 5 of 7] Site status under Old and New Rule when both are closed. Viewed as Filter Level 2.



Note that the sites are closed under both the old and new rule (2nd and 3rd columns) and either waiting (W) or in the count down period (D) (8th and 9th columns).

While you are still in Step 5 of 7, **set the Filter level to Level 3 (Figure 19).**

Figure 19. : [Step 5 of 7] Simulated Closures. Critical Period Site status under Old and New Rule. Viewed at Filter Level 3.



NOTE: Critical Period = Sites closed under the Old Rule but Open under the New Rule.
Figure 19 showing step 5 of 7 at filter level 3 is displaying critical data relative to any analysis designed to change a rainfall closure rule. These show the Critical Samples that must be taken (when the site is closed under the Old Rule, but would be open under the hypothetical New Rule. The screen displays the status of the site as closed under the old rule, but open under the hypothetical new rule (2^{nd} and 3^{rd} column) and the status the sites as either in a waiting period, countdown period or grace period under both rules (8^{th} and 9^{th} columns).

Navigating SIMULATION OUTPUT: Simulated Closures [Step 5 of 7]

[Help] Displays help message.

[Print] Prints the data sheet.

< [Filter Level] > Allows filtration of data to show specific aspects of the analysis.

[Export] Allows you to export files to an Excel spreadsheet.

[Exit] Terminates Aquarius program.

[Previous] Takes you back to the previous screen showing the input tables for the Old Rules and hypothetical New Rules.

[Next] Shows the real fecal coliform samples under simulated closures

Press Next to display Step 6 of 7, the real fecal coliform samples under simulated closures**.**

Press **Next**.

Figure 20a shows the OUTPUT: Samples During Simulated Closures [Step 6 of 7]. It displays all records of real fecal coliform records under the old and new Rules, and viewed with screen scrolled to the right. It is also viewed at Filter Level = 0.

Figure 20a displays the Region, Sites, and the open or closed status under the Oldstatus and the Newstatus, the Date and Time, the Fecal Coliform Most Probable Number, Sample Type, whether the sample was taken under Adverse Condition, and a comments section.

If you move the screen to the right as seen in Figure 20b, it further displays the Serial Number and Parent Number of the sample type, the Agency Code and the County Code. The last two columns were calculated during the analysis and consist of the calculated fecal coliform (Fcalc) and the Log of the calculated fecal coliform (Fclog). The last two columns are calculated on the fly.

Figure 20a. Step 6 of 7, OUTPUT: Samples During Simulated Closures. All Records of Real FC under Old and New Rule. Viewed with screen scrolled to the left. Viewed at Filter Level = 0.

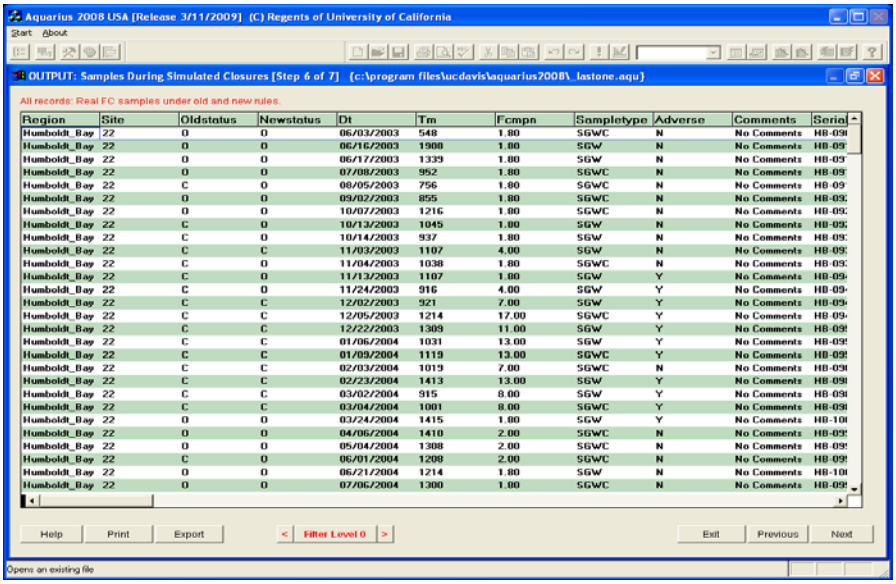
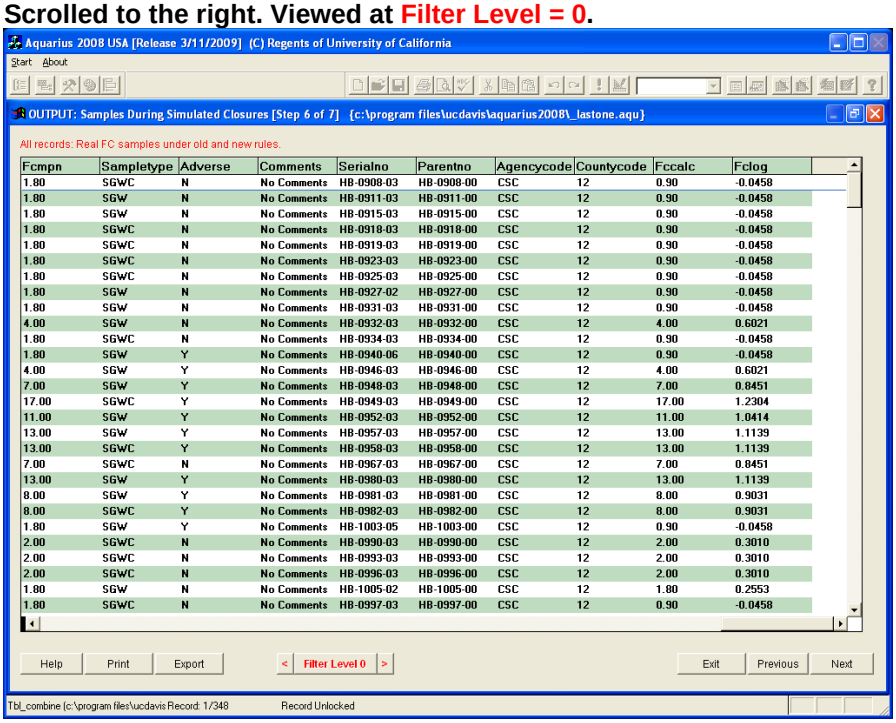


Figure 20b. Step 6 of 7, OUTPUT: Samples During Simulated Closures. All Records of Real FC under Old and New Rule, viewed with screen Scrolled to the right. Viewed at Filter Level = 0.



Fcmpn	Sampletype	Adverse	Comments	Serialno	Parentno	Agencycode	Countycode	Fccalc	Fclog
1.80	SGWC	N	No Comments	HB-0908-03	HB-0908-00	CSC	12	0.90	-0.0458
1.80	SGW	N	No Comments	HB-0911-03	HB-0911-00	CSC	12	0.90	-0.0458
1.80	SGW	N	No Comments	HB-0915-03	HB-0915-00	CSC	12	0.90	-0.0458
1.80	SGWC	N	No Comments	HB-0918-03	HB-0918-00	CSC	12	0.90	-0.0458
1.80	SGWC	N	No Comments	HB-0919-03	HB-0919-00	CSC	12	0.90	-0.0458
1.80	SGWC	N	No Comments	HB-0923-03	HB-0923-00	CSC	12	0.90	-0.0458
1.80	SGWC	N	No Comments	HB-0925-03	HB-0925-00	CSC	12	0.90	-0.0458
1.80	SGW	N	No Comments	HB-0927-02	HB-0927-00	CSC	12	0.90	-0.0458
1.80	SGW	N	No Comments	HB-0931-03	HB-0931-00	CSC	12	0.90	-0.0458
4.00	SGW	N	No Comments	HB-0932-03	HB-0932-00	CSC	12	4.00	0.6021
1.80	SGWC	N	No Comments	HB-0934-03	HB-0934-00	CSC	12	0.90	-0.0458
1.80	SGW	Y	No Comments	HB-0940-06	HB-0940-00	CSC	12	0.90	-0.0458
4.00	SGW	Y	No Comments	HB-0946-03	HB-0946-00	CSC	12	4.00	0.6021
7.00	SGW	Y	No Comments	HB-0948-03	HB-0948-00	CSC	12	7.00	0.8451
17.00	SGWC	Y	No Comments	HB-0949-03	HB-0949-00	CSC	12	17.00	1.2304
11.00	SGW	Y	No Comments	HB-0952-03	HB-0952-00	CSC	12	11.00	1.0414
13.00	SGW	Y	No Comments	HB-0957-03	HB-0957-00	CSC	12	13.00	1.1139
13.00	SGWC	Y	No Comments	HB-0958-03	HB-0958-00	CSC	12	13.00	1.1139
7.00	SGWC	N	No Comments	HB-0967-03	HB-0967-00	CSC	12	7.00	0.8451
13.00	SGW	Y	No Comments	HB-0980-03	HB-0980-00	CSC	12	13.00	1.1139
8.00	SGW	Y	No Comments	HB-0981-03	HB-0981-00	CSC	12	8.00	0.9031
8.00	SGWC	Y	No Comments	HB-0982-03	HB-0982-00	CSC	12	8.00	0.9031
1.80	SGW	Y	No Comments	HB-1003-05	HB-1003-00	CSC	12	0.90	-0.0458
2.00	SGWC	N	No Comments	HB-0990-03	HB-0990-00	CSC	12	2.00	0.3010
2.00	SGWC	N	No Comments	HB-0993-03	HB-0993-00	CSC	12	2.00	0.3010
2.00	SGWC	N	No Comments	HB-0996-03	HB-0996-00	CSC	12	2.00	0.3010
1.80	SGW	N	No Comments	HB-1005-02	HB-1005-00	CSC	12	1.80	0.2553
1.80	SGWC	N	No Comments	HB-0997-03	HB-0997-00	CSC	12	0.90	-0.0458

Practice viewing the data screen at different filter levels.

Navigating [Step 6 of 7]

[Help] Displays help message.

[Print] Prints data sheet.

[Export] Exports database to an Excel file.

[Exit] Terminates Aquarius program.

< [Filter Level] > Allows filtration of data to show specific aspects of the analysis.

[Previous] Takes you back to the previous screen showing simulated closures using real rainfall data under the Old Rules and the hypothetical New Rules.

[Next] Shows the statistical analysis.

Filter Levels

Filter Level 0: Default Screen - All records, and showing Open and Closed status of site under Old and New Rules.

Filter Level 1: Shows the sites under the Old and New Rules, when the sites are open under both rules.

Filter Level 2: Shows the sites under the Old and New Rules, when the sites are closed under both rules.

Filter Level 3: Shows the sites under the Old and New Rules, when the site is closed under the old rule, but open under the hypothetical new rule. This is the Critical Period.

Critical Period: In the Help menu, press the Critical Period button. This will reveal a diagram (Figure 21), demonstrating the critical period in which the fecal coliform samples must be taken to perform the statistical analysis that could support a change in rainfall closure rules. The Critical Period is when the site is closed under the Old Rules, but opens during the proposed New Rules, or vice versa.

Figure 21. Diagrammatic representation of fecal coliform sampling during the Critical Period in a scenario that would relax the rainfall closure rule.

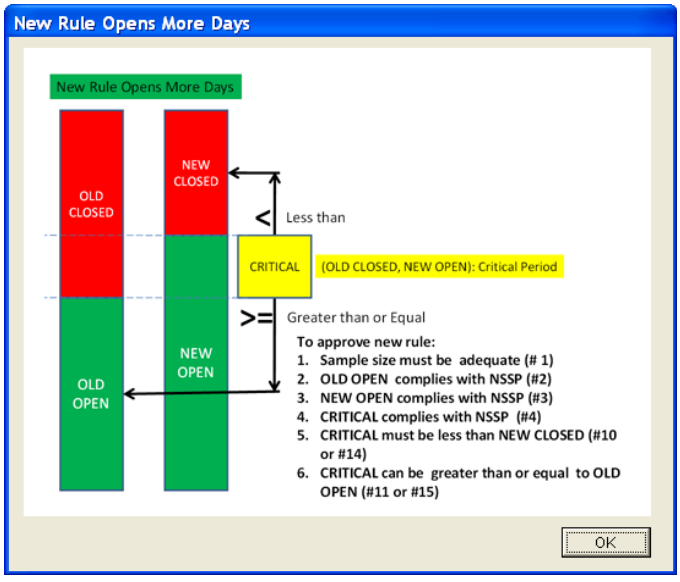


Figure 21 shows a scenario in which the hypothetical New Rule opens more days representing an attempt to relaxed rule. For example, if you were to relax the Old Rule by changing the rainfall closure from a 3-days closure after a 1.0-inche rainfall, to closing the area for 3-days after a 1.2-inch rainfall. Critical Period samples are depicted by the yellow box, which represent the area within the

dotted lines extending through the two bars in which the area is closed under the Old Rule (Red), but would be open under the hypothetical New Rule (Green). Disregard the other information in the diagram for now, as that information will be applied to the result sections in the final statistical report.

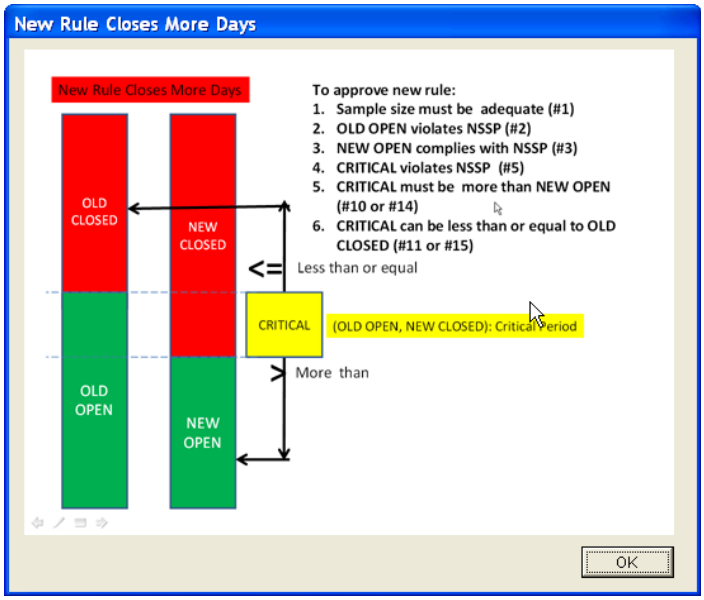
Note: Most water samples for fecal coliform that are taken in the field are taken as close as possible after a harvest site is reopened following a rainfall closure period. These are Compliance Samples, which are taken to verify if the harvest site is still in compliance with the NSSP standards for shellfish harvest. Compliance samples have value as they are a check to see if the rainfall closure rule that is being applied is still valid.

In order to obtain a measure of statistical validation to change a rainfall closure rule, a statistical analysis should be preformed on samples taken during the Critical Period, when the harvest site is closed under the Old Rule, but would be open under the hypothetical New Rule, or vice versa. These samples are Critical Period Samples.

Two examples will suffice as an illustration of the value of Critical Samples. If a harvest site is regulated under a 1-inch rainfall in 24-hours for a 3-day closure, and substantial progress has been made to improve the surrounding watershed in terms of reducing levels of detectable fecal coliform flowing into the bay, selfish growers may seek validation to relax the rainfall closure rule to 1.2-inches in 24-hours with a 3-day closure. This is an example of employing the concept illustrated in Figure 21 in which samples are taken during the Critical Period in which the site was closed under the Old Rule (1-inch), but would be relaxed, or open under the hypothetical New Rule (1.2-inches). This would, in effect, open more days for harvest. As part of the analysis, parametric T-tests are performed to determine if there is a significant difference in the samples taken during the critical period. If there are no significant differences, and other statistical parameters meet NSSP standards, the site could be considered for a relaxation of the closure rule. If there are significant differences in the samples, or other statistical parameters described later are not met, the criteria for relaxation fail.

Conversely, if the agency suspects that a shellfish area has deteriorated due to an increase in fecal coliform appearing in the watershed, test can be performed to stiffen the rainfall closure rule. An example would be to change the closure rule from a 3-day closure after a 1.0-inch rainfall in 24-hours to a 3-day closure after a 0.75-inch rainfall in 24-hours. An example of stiffening the rainfall closure rule can be seen in Figure 22, in which the hypothetical New Rule would close the site for exceeding a lower level of rainfall in 24-hours. Again, disregard the additional information in the diagram, as that information applies to the final statistical report. If there are no significant differences, and other statistical parameters meet NSSP standards, the site is considered for stiffening of the closure rule. If there are significant differences in the samples, or other statistical parameters described later are not met, the criteria for stiffening the rule fail.

Figure 22. Diagrammatic representation of fecal coliform sampling during the Critical Period in a scenario that would make the rainfall closure rule more restrictive.



Press Next to display the final statistical data report.

The final statistical report will appear as either [Step 7a of 7] or [Step 7b of 7].

[Step 7a of 7] appears if the results of the statistical analysis are positive, that is showing no significant differences in the Critical Period samples, and all of the statistical parameters meet the NSSP criteria.

[Step 7b of 7] appears if the results of the statistical analysis are negative, that is showing significant differences in the Critical Period samples, and one or more of the statistical parameters do not meet the NSSP criteria.

OUTPUT: Statistical Analysis [Step 7a of 7]

Aquarius' final statistical report has 22 potential section displays.

Section 1. This section provides a summary of the number of days samples were taken when the site was both open and closed under the two rules, and a summary of the number of samples taken during those days. It also provides a summary of the number of samples taken during the Critical Period (Old Closed, New Open).

Sections 2 - 7. These sections provide *descriptive statistics*, determining whether or not specific samples taken are in compliance with NSSP standards. For example, to meet the NSSP criteria the Geometric Mean of samples taken must be less than 14 and the Estimated 90^{th} percentile must be less than 49 for the 3-tube Test.

Sections 8 - 11. These sections are the results of *parametric, T-tests* performed on specific samples in specific categories to determine if there are significant differences between the sample sets.

Sections 12 - 15. These sections provide Non Parametric, Wilcoxon Rank Sum Test, which are used if the user believes that the samples are not normally distributed.

Sections 16 - 22. Informational sections.

The Aquarius v. 2 program potentially generates one of four potential report forms. The report forms fall into two major groups, Step 7a reports in which the hypothetical new rules relaxes the rainfall closure rule and opens more days, or Step 7b reports in which the hypothetical new rules stiffens the rainfall closure rule and opens fewer days. Both the Step 7a and Step 7b reports have a positive format in which the statistical analysis supports the proposed changes; and a negative format in which the statistical analysis do not support the proposed changes. In summary, the four report options are as follows:

Step 7a (Positive Format) Scenario in which the hypothetical new rule opens more days and is supported by the statistical analysis.

Step 7a (Negative Format) Scenario in which the hypothetical new rule opens more days and is not supported by the statistical analysis.

Step 7b (Positive Format) Scenario in which the hypothetical new rule opens fewer days and is supported by the statistical analysis.

Step 7b (Negative Format) Scenario in which the hypothetical new rule opens fewer days and is not supported by the statistical analysis.

The report can be refined by setting the appropriate Filter Level, thereby providing a view of all sections, including those sections that do not apply to an analysis, or the appropriate sections that apply to either a parametric or non parametric application. For Example:

Filter Level 0 Displays all sections, whether they apply to a specific, hypothetical scenario, or not.

Filter Level 1 Displays NSSP and Parametric applications (Sections).

Filter Level 2 Displays NSSP and Non-Parametric applications (Sections).

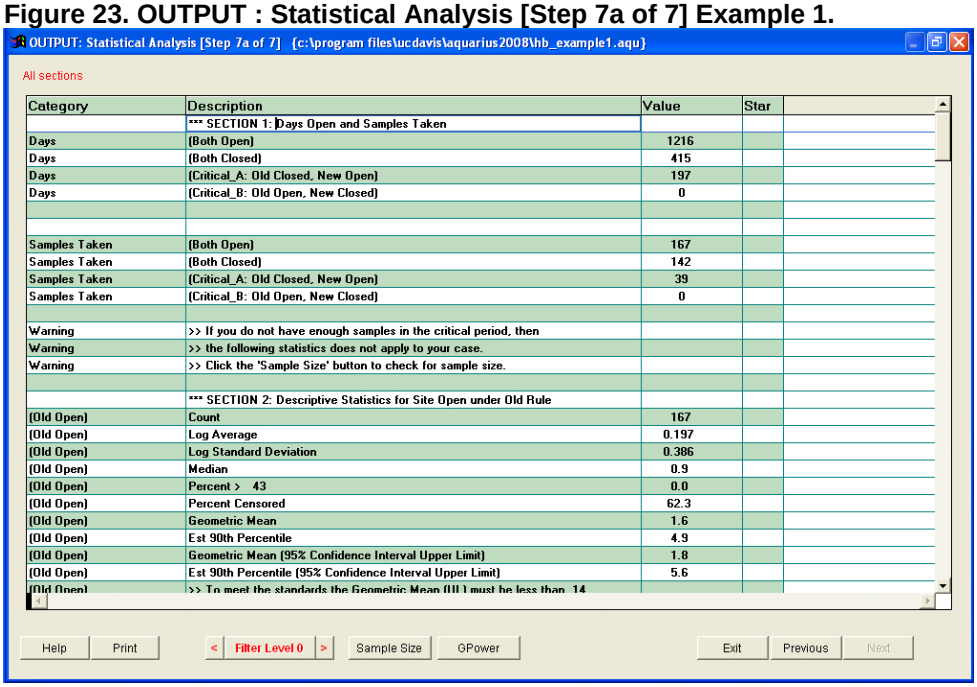
Filter Lever 3 Displays NSSP Sections only.

In our first example, which is a parametric application, we will set the filter level at 0 to display all sections to provide a general overview of the statistical report. The report will also provide information that instructs the user as to what sections are not part of the test, but provides an opportunity to view the anatomy of each section.

To activate the appropriate view of a parametric application, the user would set the filter level at Filter Lever 1. However, in this first example, we will set the filter level at Filter Level 0 and review the anatomy of the full report.

Set the display to Filter Level 0

Figure 23. OUTPUT : Statistical Analysis [Step 7a of 7] Example 1.



Category	Description	Value	Star
	*** SECTION 1: Days Open and Samples Taken		
Days	(Both Open)	1216	
Days	(Both Closed)	415	
Days	(Critical_A: Old Closed, New Open)	197	
Days	(Critical_B: Old Open, New Closed)	0	
Samples Taken	(Both Open)	167	
Samples Taken	(Both Closed)	142	
Samples Taken	(Critical_A: Old Closed, New Open)	39	
Samples Taken	(Critical_B: Old Open, New Closed)	0	
Warning	>> If you do not have enough samples in the critical period, then		
Warning	>> the following statistics does not apply to your case.		
Warning	>> Click the 'Sample Size' button to check for sample size.		
	*** SECTION 2: Descriptive Statistics for Site Open under Old Rule		
(Old Open)	Count	167	
(Old Open)	Log Average	0.197	
(Old Open)	Log Standard Deviation	0.386	
(Old Open)	Median	0.9	
(Old Open)	Percent > 43	0.0	
(Old Open)	Percent Censored	62.3	
(Old Open)	Geometric Mean	1.6	
(Old Open)	Est 90th Percentile	4.9	
(Old Open)	Geometric Mean (95% Confidence Interval Upper Limit)	1.8	
(Old Open)	Est 90th Percentile (95% Confidence Interval Upper Limit)	5.6	
(Old Open)	>> To meet the standards the Geometric Mean (UL) must be less than 14		

Figure 23 is a whole-screen shot of the analytical report. To view the entire report requires scrolling down through the various sections. The menu bar at the bottom contains menu buttons for functions that we have covered before, such as Help, Print, Exit and Previous, which are by now apparent. We will address the remaining buttons, Filter Level, Sample Size and GPower, as we proceed through the examples. For ease of instruction, we will use screen shots of individual sections.

Figure 23 provides a summary of the sample days and the status of the sites and the relationship of the samples taken, which are used calculations of sections that follow.

Figure 23. Section 1 Statistical Analysis [Step 7a of 7] Example 1.

Category	Description	Value	Star
	*** SECTION 1: Days Open and Samples Taken		
Days	(Both Open)	1216	
Days	(Both Closed)	415	
Days	(Critical_A: Old Closed, New Open)	197	
Days	(Critical_B: Old Open, New Closed)	0	
Samples Taken	(Both Open)	167	
Samples Taken	(Both Closed)	142	
Samples Taken	(Critical_A: Old Closed, New Open)	39	
Samples Taken	(Critical_B: Old Open, New Closed)	0	
Warning	>> If you do not have enough samples in the critical period, then		
Warning	>> the following statistics does not apply to your case.		
Warning	>> Click the 'Sample Size' button to check for sample size.		

Section 1. Provides a summary of days when both sites are open under both the Old Rule and the New Rule, and when both sites are closed under the Old Rule and the New Rule;

The number of days appearing in Critical A: (Old Closed, New Open (The Critical Period);

The number of days appearing in Critical B: (Old Open, New Closed;

A summary of samples taken when both rules opened the site;

A summary of samples taken when both rules closed the site;

A summary of the number of samples taken during Critical A: (Old Closed, New Open) (The Critical Period);

And, a summary of the number of samples taken during Critical B: (Old Open, New Closed).

There is also a warning that if you do not have sufficient Critical Period samples, then the statistical analysis that follows is not valid. Sample size calculations will

be covered later in the manual, as the sample size analysis applies to specific sections in the total analysis.

Descriptive Statistics

As stated earlier, Sections 2 through 7 are descriptive statistics to determining whether or not specific samples taken are in compliance with NSSP standards. Figure 24 shows the results of Section 2, which is a statistical determination to check if the Geometric Mean of samples taken during Old Open are less than the NSSP standard of 14 and the Estimated 90th percentile must be less than 43 for the 5-tube Test.

Figure 24. Section 2 Statistical Analysis [Step 7a of 7] Example 1.

	*** SECTION 2: Descriptive Statistics for Site Open under Old Rule		
(Old Open)	Count	167	
(Old Open)	Log Average	0.197	
(Old Open)	Log Standard Deviation	0.386	
(Old Open)	Median	0.9	
(Old Open)	Percent > 43	0.0	
(Old Open)	Percent Censored	62.3	
(Old Open)	Geometric Mean	1.6	
(Old Open)	Est 90th Percentile	4.9	
(Old Open)	Geometric Mean (95% Confidence Interval Upper Limit)	1.8	
(Old Open)	Est 90th Percentile (95% Confidence Interval Upper Limit)	5.6	
(Old Open)	>> To meet the standards the Geometric Mean (UL) must be less than 14		
(Old Open)	>> and not more than 10% of the samples shall exceed 43		
(Old Open)	>> and the Est 90th Percentile (UL) must be less than 43 for 5-tube Test.		
(Old Open)	>> This data set meets the above limits.		***
(Old Open)	>> This test supports the rainfall rule change.		***

The Geometric Mean is 1.6 and the Estimated 90th percentile is 4.9, therefore the dataset for Old Open meets the NSSP fecal coliform water quality standard and supports the New Rule. In addition, the more conservative test, the Estimated 90th percentile, 95% Confidence Interval Upper Limit is 5.6, and also meets the criteria. **Note:** The three stars in the far right hand column are to draw your attention to the section summary.

Figure 25, below, shows the results of Section 3, which is a statistical determination to check if the Geometric Mean of samples taken during New Open are less than the NSSP standard of 14 and the Estimated 90th percentile must be less than 43 for the 5-tube Test.

Figure 25. Section 3 Statistical Analysis [Step 7a of 7] Example 1.

	*** SECTION 3: Descriptive Statistics for Site Open under New Rule		
(New Open)	Count	206	
(New Open)	Log Average	0.251	
(New Open)	Log Standard Deviation	0.409	
(New Open)	Median	0.9	
(New Open)	Percent > 43	0.0	
(New Open)	Percent Censored	55.8	
(New Open)	Geometric Mean	1.8	
(New Open)	Est 90th Percentile	5.9	
(New Open)	Geometric Mean (95% Confidence Interval Upper Limit)	2.0	
(New Open)	Est 90th Percentile (95% Confidence Interval Upper Limit)	6.8	
(New Open)	>> To meet the standards the Geometric Mean (UL) must be less than 14		
(New Open)	>> and not more than 10% of the samples shall exceed 43		
(New Open)	>> and the Est 90th Percentile (UL) must be less than 43 for 5-tube Test.		
(New Open)	>> This data set meets the above limits.		***
(New Open)	>> This test supports the rainfall rule change.		***

The Geometric Mean is 1.8 and the Estimated 90th percentile is 5.9, therefore the dataset for New Open meets the NSSP fecal coliform water quality standard and supports the New Rule. In addition, the more conservative test, the Estimated 90th percentile, 95% Confidence Interval Upper Limit is 6.8, and also meets the criteria.

Figure 26 shows the results of Section 4, which is a statistical determination to check if the Geometric Mean of samples taken during Old Closed, New Open (Critical Samples, Figure 21) are less than the NSSP standard of 14 and the Estimated 90th percentile must be less than 43 for the 5-tube Test.

Figure 26. Section 4 Statistical Analysis [Step 7a of 7] Example 1.

	*** SECTION 4: Descriptive Statistics for Critical Period A		
(Old Closed, New Open)	Count	39	
(Old Closed, New Open)	Log Average	0.483	
(Old Closed, New Open)	Log Standard Deviation	0.426	
(Old Closed, New Open)	Median	4.0	
(Old Closed, New Open)	Percent > 43	0.0	
(Old Closed, New Open)	Percent Censored	28.2	
(Old Closed, New Open)	Geometric Mean	3.0	
(Old Closed, New Open)	Est 90th Percentile	10.7	
(Old Closed, New Open)	Geometric Mean (95% Confidence Interval Upper Limit)	4.1	
(Old Closed, New Open)	Est 90th Percentile (95% Confidence Interval Upper Limit)	15.3	
(Old Closed, New Open)	>> To meet the standards the Geometric Mean (UL) must be less than 14		
(Old Closed, New Open)	>> and not more than 10% of the samples shall exceed 43		
(Old Closed, New Open)	>> and the Est 90th Percentile (UL) must be less than 43 for 5-tube Test.		
(Old Closed, New Open)	>> This data set meets the above limits.		***
(Old Closed, New Open)	>> This test supports the rainfall rule change.		***

The Geometric Mean is 3.0 and the Estimated 90th percentile is 10.7, therefore the dataset for New Open meets the NSSP fecal coliform water quality standard and supports the New Rule. In addition, the more conservative test, the Estimated 90th percentile, 95% Confidence Interval Upper Limit is 15.3, and also meets the criteria.

Figure 27 shows the results of Section 5, which is a statistical determination to check if the Geometric Mean of samples taken during Old Open, New Closed is less than the NSSP standard of 14 and the Estimated 90th percentile must be less than 43 for the 5-tube Test.

Figure 27. Section 5 Statistical Analysis [Step 7a of 7] Example 1.

	*** SECTION 5: Descriptive Statistics for Critical Period B		
(Old Open, New Closed)	Count	0	
(Old Open, New Closed)	Log Average	0.000	
(Old Open, New Closed)	Log Standard Deviation	0.000	
(Old Open, New Closed)	Median	0.0	
(Old Open, New Closed)	Percent > 43	0.0	
(Old Open, New Closed)	Percent Censored	0.0	
(Old Open, New Closed)	Geometric Mean	0.0	
(Old Open, New Closed)	Est 90th Percentile	0.0	
(Old Open, New Closed)	Geometric Mean (95% Confidence Interval Upper Limit)	0.0	
(Old Open, New Closed)	Est 90th Percentile (95% Confidence Interval Upper Limit)	0.0	
(Old Open, New Closed)	>> To meet the standards the Geometric Mean (UL) must be less than 14		
(Old Open, New Closed)	>> and not more than 10% of the samples shall exceed 43		
(Old Open, New Closed)	>> and the Est 90th Percentile (UL) must be less than 43 for 5-tube Test.		
(Old Open, New Closed)	>> This data set meets the above limits.		***
(Old Open, New Closed)			***

Figure 27 shows the results of Section 5, which is a statistical determination to check if the Geometric Mean of samples taken during Old Open, New Closed is less than the NSSP standard of 14 and the Estimated 90th percentile must be less than 43 for the 5-tube Test. Notice that all the results are 0.0. This is logical, since we are relaxing the rainfall closure rules, resulting in more days open. When more days are open, it is impossible to have samples when more days are closed. Section 5 is ignored when rainfall closure rules are relaxed.

Figure 28 shows the results of Section 6, which is a statistical determination to check if the Geometric Mean of samples taken during Old Closed is less than the NSSP standard of 14 and the Estimated 90th percentile must be less than 43 for the 5-tube Test. Although the Old Closed does not meet the NSSP standard, the Old Closed dataset is not used in new rule approval.

Figure 28. Section 6 Statistical Analysis [Step 7a of 7] Example 1.

	*** SECTION 6: Descriptive Statistics for Site Closed under Old Rule		
(Old Closed)	Count	181	
(Old Closed)	Log Average	0.843	
(Old Closed)	Log Standard Deviation	0.566	
(Old Closed)	Median	8.0	
(Old Closed)	Percent > 43	9.9	
(Old Closed)	Percent Censored	13.3	
(Old Closed)	Geometric Mean	7.0	
(Old Closed)	Est 90th Percentile	37.0	
(Old Closed)	Geometric Mean (95% Confidence Interval Upper Limit)	8.4	
(Old Closed)	Est 90th Percentile (95% Confidence Interval Upper Limit)	44.8	
(Old Closed)	>> To meet the standards the Geometric Mean (UL) must be less than 14		
(Old Closed)	>> and not more than 10% of the samples shall exceed 43		
(Old Closed)	>> and the Est 90th Percentile (UL) must be less than 43 for 5-tube Test.		
(Old Closed)	>> This data set DOES NOT meet the above limits.		***
(Old Closed)	>> Not used in new rule approval.		***

Figure 29 shows the results of Section 7, which is a statistical determination to check if the Geometric Mean of samples taken during New Closed is less than the NSSP standard of 14 and the Estimated 90th percentile must be less than 43 for the 5-tube Test.

Figure 29. Section 7 Statistical Analysis [Step 7a of 7] Example 1.

	*** SECTION 7: Descriptive Statistics for Site Closed under New Rule		
(New Closed)	Count	142	
(New Closed)	Log Average	0.941	
(New Closed)	Log Standard Deviation	0.561	
(New Closed)	Median	9.5	
(New Closed)	Percent > 43	12.7	
(New Closed)	Percent Censored	16.9	
(New Closed)	Geometric Mean	8.7	
(New Closed)	Est 90th Percentile	45.7	
(New Closed)	Geometric Mean (95% Confidence Interval Upper Limit)	10.8	
(New Closed)	Est 90th Percentile (95% Confidence Interval Upper Limit)	56.8	
(New Closed)	>> To meet the standards the Geometric Mean (UL) must be less than 14		
(New Closed)	>> and not more than 10% of the samples shall exceed 43		
(New Closed)	>> and the Est 90th Percentile (UL) must be less than 43 for 5-tube Test.		
(New Closed)	>> This data set DOES NOT meet the above limits.		***
(New Closed)	>> Not used in new rule approval.		***

The Geometric Mean is 8.7 and the Estimated 90 percentile is 45.7 and meets the NSSP standards, however the Estimated 90th percentile, 95% Confidence Interval Upper Limit is 56.8 and exceeds the limit. We will return to the 95% Confidence Interval Upper Limit later to discuss the value of this tool in decision making processes. However, note that the calculations for (New Closed) are not used in new rule approval and can be ignored in this analysis. The New Closed would naturally exceed the NSSP standards.

Parametric T-Tests

Sections 8 through 11 show the results of parametric T-tests used when datasets are normally distributed. Parametric T-tests are preferred over regression analysis as they are designed to determine if there are significant differences between data sets, in this case an old rule and a hypothetical new rule. If there are no significant differences between the two data sets, then the new rule can be adopted, if other statistical parameters related to NSSP standards and adequate sample sizes are met. The application of the T-test is believed to be superior to regression analysis (RA) as RA depends on trends and interpretation of trends to make a final determination.

Figure 30 displays T-test results from a comparison of Old Open (n1) against Old Closed (n2).

Figure 30. Section 8 Statistical Analysis [Step 7a of 7] Example 1.

	*** SECTION 8: Parametric Test Comapring (Old Open) vs (Old Closed)		
COMPARISON	(Old Open) VS (Old Closed)		
T-Test	n1	167	
T-Test	n2	181	
T-Test	Lower t sub a	-1.960	
T-Test	Computed t sub c	-12.514	
T-Test	Upper t sub b	1.960	
T-Test	Alpha level	0.050	
T-Test	Degree of freedom	321	
T-Test	>> T-Test IS significant at Alpha = 0.050		
T-Test	>> (Old Open) IS LESS THAN (Old Closed)		***
T-Test	>> Required sample size for EACH group is 16.		
T-Test	>> This data set meets the required sample size.		***
T-Test	>> Not used in new rule approval.		***
T-Test	The following values are useful for calculating required sample size:		
T-Test	n1= 167, Avg1= 0.197, Std1= 0.386, n2= 181, Avg2= 0.843, Std2= 0.566		
T-Test	Pooled Std= 0.487, Diff Avg = 0.646, Effect Size d = 1.000		

Although the third set of stars in the last column tells you that Section 8 is not used in new rule approval, its results can be instructional for the rest of the parametric T-test sections. The results show that the T-test is significant at Alpha = 0.05 and that (Old Open) is less than (Old Closed). Also, that the required sample size for each group (n1 and n2) is 16. In our scenario, n1 = 167 samples and n2 = 181 samples.

At the bottom of the section 8 screen are numerical values that are used in calculating required sample size. These values will be used later in the manual in calculating required sample size using the GPower program.

This provides a useful first look at Sample Size. Aquarius ver. 2.0 has a built in sample size statistical program that can be activated by pressing the Sample Size button in the menu line at the bottom of the screen. Sample Size is one of two statistical programs used by Aquarius to determine adequacy of sample size.

Press Sample Size

The Sample Size dialog box appears displaying the first of two sample size statistical programs.

Figure 31. Sample Size Screen 1 Example 1.

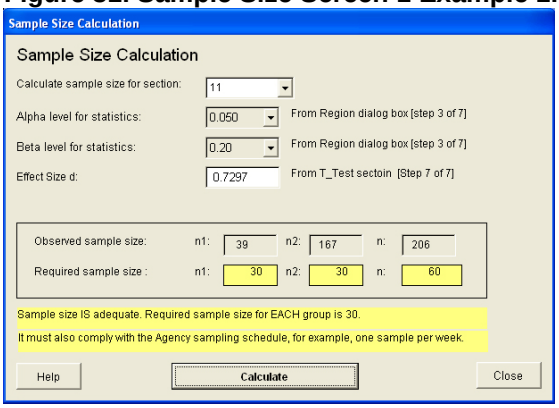


Figure 31 is the dialog box for the built in Sample Size statistical program. The sample size is calculated using the T-test sections of the Statistical output [Step 7 of 7: sections 8, 9, 10, and 11]. The sample size must be adequate for all these four sections. It must also comply with the Agency sampling schedule, for example one sample per week.

The range of accepted sample size is from 15 to 100 samples per group, or 30 to 200 total samples. Aquarius enforces this range check on the sample size calculation algorithm.

Note: As a rule of thumb, if you are using non-parametric tests, you must add 15% more to the calculated sample size.

To calculate the required sample size, the following steps are employed.

Using the dialog box, select either section 8, 9, 10, or 11.Because we are analyzing Section 8, select 8 in the drop down menu for calculate sample size. The program automatically loads the value for 'Effective Size D' from the T-test section of the selected section. The Effect Size D is calculated by dividing the difference between two means by the pooled standard deviation. The valid range is between 0.30 and 1.00. The program allows other values, but the statistical report enforces these minimum and maximum limits for the Effect Size D.

For 'Alpha level for statistics' accept the Alpha level that you entered in the Region dialog box [Step 3 of 7].

For 'Beta level for statistics' accept the Beta level you entered in the Region dialog box [Step 3 of 7].

For 'Observed Sample size', accept the values loaded by the program. The Code n1 denotes sample size for group 1. The code n2 denotes sample size for group 2. The code n denotes total sample size (n1+n2).

Click the 'Calculate' button and the program calculates the required sample size for each group as well as the total sample size. The program then compares the observed sample size with required sample size and displays a message stating if the sample size is adequate or not (Figure 32).

Figure 32 Sample Size Screen 2 Example 1.

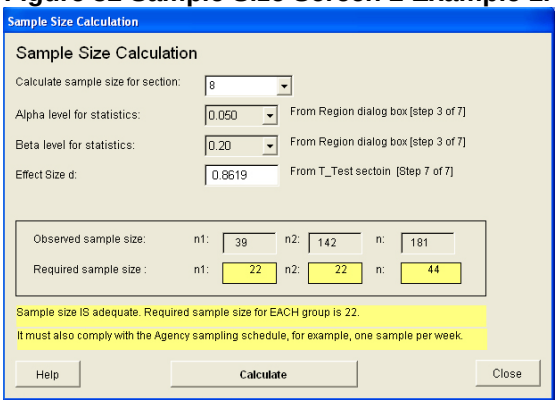


Figure 32 Displays the observed sample size for n1 (167) and n2 (181) and n (total = 348). It also states that the sample size is adequate, and the required sample size for each group is 16.

Note: The sample size program also states that the sample size must also comply with the Agency sampling schedule, for example one sample per week. If it does not comply then it is not adequate, even if the calculated sample size deemed adequate.

Reference: The sample size calculation is based on the method developed in the St George's University of London. For more information please visit: http://www.sgul.ac.uk/index.cfm?DD25D103-C079-6609-F78D-BC005970DCD9

The formula for the sample size for comparison of 2 means (2-sided) is as follows:

n = [A + B]^2 * 2 * SD^2 / DIFF^2

Where n = Sample size required in each of the trial's two groups. Therefore the total sample size is double this value.

SD = Pooled standard deviation
SD = SQRT(((n1-1)*nVar1 +(n2-1)*nVar2)/(n1+n2))

where nVar1 = nStd1 ^ 2
nVar2 = nStd2 ^ 2
n1 and n2 are observed sample sizes in each group

DIFF = Size of difference between two means
DIFF = ABS(Avg1 – Avg2)

A = Depends on desired alpha level (see table)

B = Depends on desired power (see table). Power = 1 - Beta

Table of values for A

Alpha	A
0.10	1.64
0.05	1.96
0.025	2.23
0.01	2.58

Table of values for B

Power	B
0.80	0.84
0.90	1.28
0.96	1.64

Press Close to terminate the Sample size program.

Aquarius 2.0 also has access to a second, more powerful sample size statistical program called GPower. The authors have permission to install GPower into Aquarius from the creators of the program. GPower is covered later in the manual.

Figure 33 displays T-test results from a comparison of New Open (n1) against New Closed (n2).

Figure 33. Section 9 Statistical Analysis [Step 7a of 7] Example 1.

	*** SECTION 9: Parametric Test Comapring (New Open) vs (New Closed)		
COMPARISON	(New Open) VS (New Closed)		
T-Test	n1	206	
T-Test	n2	142	
T-Test	Lower t sub a	-1.960	
T-Test	Computed t sub c	-12.552	
T-Test	Upper t sub b	1.960	
T-Test	Alpha level	0.050	
T-Test	Degree of freedom	242	
T-Test	>> T-Test IS significant at Alpha = 0.050		
T-Test	>> (New Open) IS LESS THAN (New Closed)		***
T-Test	>> Required sample size for EACH group is 16.		
T-Test	>> This data set meets the required sample size.		***
T-Test	>> Not used in new rule approval.		***
T-Test	The following values are useful for calculating required sample size:		
T-Test	n1= 206, Avg1= 0.251, Std1= 0.409, n2= 142, Avg2= 0.941, Std2= 0.561		
T-Test	Pooled Std= 0.475, Diff Avg = 0.691, Effect Size d = 1.000		

The third set of stars in the last column indicate the line that tells you that Section 9 is also not used in new rule approval. However, as a review of the general concepts, results show that the T-test is significant at Alpha = 0.05 and that (New Open) is less than (New Closed). Also, that the required sample size for each group (n1 and n2) is 16. In our scenario, n1 = 206 samples and n2 = 142 samples. If the Sample Size program is employed, results show that the sample size results in Section 9 match the results in the data sheet readout and the required sample size for n1 and n2 are 16 each, for a total of 32. The sample sizes for n1 and n2 are 206 and 142 respectively. Again, the numerical values at the bottom of the section are used in calculating required sample size.

Figure 34 displays T-test results from a comparison of Old Closed, New Open (n1) against New Closed (n2).

Figure 34. Section 10 Statistical Analysis [Step 7a of 7] Example 1.

	*** SECTION 10: Parametric Test Comparing (Critical A) vs (New Closed)		
COMPARISON	(Old Closed, New Open) VS (New Closed)		
T-Test	n1	39	
T-Test	n2	142	
T-Test	Lower t sub a	-1.990	
T-Test	Computed t sub c	-5.534	
T-Test	Upper t sub b	1.990	
T-Test	Alpha level	0.050	
T-Test	Degree of freedom	80	
T-Test	>> T-Test IS significant at Alpha = 0.050		
T-Test	>> (Old Closed, New Open) IS LESS THAN (New Closed)		***
T-Test	>> Required sample size for EACH group is 22.		
T-Test	>> This data set meets the required sample size.		***
T-Test	>> This test supports the rainfall rule change.		***

Section 10 is an important section for analyzing the new rule, as it involves critical samples. The results show that the T-test is significant at Alpha = 0.05 and that (Old Closed, New Open) is less than (New Closed). Also, that the required sample size for each group (n1 and n2) is 22. In our scenario, n1 = 39 samples and n2 = 142 samples, and this is confirmed by the Sample Size statistical program. This test displayed in Section 10 supports the new rule, if the sampling program complies with the agency sampling schedule.

Figure 35 displays T-test results from a comparison of Old Closed, New Open (n1) against Old Open (n2).

Figure 35. Section 11 Statistical Analysis [Step 7a of 7] Example 1

	*** SECTION 11: Parametric Test Comparing (Critical A) vs (Old Open)		
COMPARISON	(Old Closed, New Open) VS (Old Open)		
T-Test	n1	39	
T-Test	n2	167	
T-Test	Lower t sub a	-2.005	
T-Test	Computed t sub c	3.838	
T-Test	Upper t sub b	2.005	
T-Test	Alpha level	0.050	
T-Test	Degree of freedom	54	
T-Test	>> T-Test IS significant at Alpha = 0.050		
T-Test	>> (Old Closed, New Open) IS GREATER THAN (Old Open)		***
T-Test	>> Required sample size for EACH group is 30.		
T-Test	>> This data set meets the required sample size.		***
T-Test	>> This test the rainfall rule change.		***
T-Test	The following values are useful for calculating required sample size:		
T-Test	n1= 39, Avg1= 0.483, Std1= 0.426, n2= 167, Avg2= 0.197, Std2= 0.386		
T-Test	Pooled Std= 0.392, Diff Avg = 0.286, Effect Size d = 0.730		

Section 11 is also an important section for supporting the new rule. The results show that the T-test is significant at Alpha = 0.05 and that (Old Closed, New Open) is greater than (Old Open). Also, that the required sample size for each group (n1 and n2) is 30. In our scenario, n1 = 39 samples and n2 = 167 samples, and this is confirmed by the Sample Size statistical program. This test displayed in Section 11 supports the new rule, if the sampling program complies with the agency sampling schedule.

Non Parametric Wilcoxon Rank Sum Test

The Wilcoxon Rank Sum test is employed when the user believes that the samples are not normally distributed. Figure 36 displays a Wilcoxon Rank Sum (WRS) test results from a comparison of Old Open (Nx) against Old Closed (Ny).

Figure 36. Section 12 Statistical Analysis [Step 7a of 7] Example 1.

	*** SECTION 12: Non-Parametric Test Comapring (Old Open) vs (Old Closed)		
COMPARISON	(Old Open) VS (Old Closed)		
NonParam	Nx	167	
NonParam	Ny	181	
NonParam	Rank Sum Rx	19402.000	
NonParam	Rank Sum Ry	41324.000	
NonParam	Computed Z	-10.388	
NonParam	Two-sided Z	1.960	
NonParam	P value	0.000	
NonParam	>> Wilcoxon Rank Sum Test IS significant at Alpha = 0.050		
NonParam	>> (Old Open) IS LESS THAN (Old Closed)		***
NonParam	>> Required sample size for EACH group is 18.		
NonParam	>> This data set meets the required sample size.		***
NonParam	>> Not used in new rule approval.		***

Although section 12 is not used to approve the new rule, we will review the results for practice. The results show that the WRS is significant at Alpha = 0.05 and that (Old Open) is less than (Old Closed). Also, that the required sample size for each group (Nx and Ny) is 18. In our scenario, Nx = 167 samples and Ny = 181 samples, and the required sample size for each is 18. However, Section 12 comparison is not used in this new rule approval.

Figure 37 displays a Wilcoxon Rank Sum (WRS) test results from a comparison of New Open (Nx) against New Closed (Ny), which is also a comparison that is not used in approving the new rule.

Figure 37. Section 13 Statistical Analysis [Step 7a of 7] Example 1.

	*** SECTION 13: Non-Parametric Test Comapring (New Open) vs (New Closed)		
COMPARISON	(New Open) VS (New Closed)		
NonParam	Nx	206	
NonParam	Ny	142	
NonParam	Rank Sum Rx	26259.000	
NonParam	Rank Sum Ry	34467.000	
NonParam	Computed Z	-10.504	
NonParam	Two-sided Z	1.960	
NonParam	P value	0.000	
NonParam	>> Wilcoxon Rank Sum Test IS significant at Alpha = 0.050		
NonParam	>> (New Open) IS LESS THAN (New Closed)		***
NonParam	>> Required sample size for EACH group is 18.		
NonParam	>> This data set meets the required sample size.		***
NonParam	>> Not used in new rule approval.		***

The results show that the WRS is significant at Alpha = 0.05 and that (New Open) is less than (New Closed). Also, that the required sample size for each group (Nx and Ny) is 18. In our scenario, Nx = 206 samples and Ny = 142 samples, and the required sample size for each is 18. However, Section 13 comparison is not used in this new rule approval.

Figure 38 displays a Wilcoxon Rank Sum (WRS) test results from a comparison of Old Closed, New Open (Nx) against New Closed (Ny).

Figure 38. Section 14 Statistical Analysis [Step 7a of 7] Example 1

	*** SECTION 14: Non-Parametric Test Comparing (Critical A) vs (New Closed)		
COMPARISON	(Old Closed, New Open) VS (New Closed)		
NonParam	Nx	39	
NonParam	Ny	142	
NonParam	Rank Sum Rx	2253.000	
NonParam	Rank Sum Ry	14218.000	
NonParam	Computed Z	-4.472	
NonParam	Two-sided Z	1.960	
NonParam	P value	0.000	
NonParam	>> Wilcoxon Rank Sum Test IS significant at Alpha = 0.050		
NonParam	>> (Old Closed, New Open) IS LESS THAN (New Closed)		***
NonParam	>> Required sample size for EACH group is 25.		
NonParam	>> This data set meets the required sample size.		***
NonParam	>> This test supports the rainfall rule change.		***

The results show that the WRS is significant at Alpha = 0.05 and that (Old Closed, New Open) is less than (New Closed). Also, that the required sample size for each group (Nx and Ny) is 25. In our scenario, Nx = 39 samples and Ny = 142 samples, and the required sample size for each is 25. Section 14 comparison supports the new rule approval using the WRS analysis.

Figure 39 displays a Wilcoxon Rank Sum (WRS) test results from a comparison of Old Closed, New Open (Nx) against Old Open (Ny).

Figure 39. Section 15 Statistical Analysis [Step 7a of 7] Example 1

	*** SECTION 15: Non-Parametric Test Comparing (Critical A) vs (Old Open)		
COMPARISON	(Old Closed, New Open) VS (Old Open)		
NonParam	Nx	39	
NonParam	Ny	167	
NonParam	Rank Sum Rx	5384.000	
NonParam	Rank Sum Ry	15937.000	
NonParam	Computed Z	4.020	
NonParam	Two-sided Z	1.960	
NonParam	P value	0.000	
NonParam	>> Wilcoxon Rank Sum Test IS significant at Alpha = 0.050		
NonParam	>> (Old Closed, New Open) IS LESS THAN (Old Open)		***
NonParam	>> Required sample size for EACH group is 35.		
NonParam	>> This data set meets the required sample size.		***
NonParam	>> This test supports the rainfall rule change.		***

The results show that the WRS is significant at Alpha = 0.05 and that (Old Closed, New Open) is less than (Old Open). Also, that the required sample size for each group (Nx and Ny) is 35. In our scenario, Nx = 39 samples and Ny = 167 samples, and the required sample size for each is 35. Section 15 comparison supports the new rule approval using the WRS analysis.

Figure 40 displays the conclusions for the Parametric T-test analysis (Section 21) and the Non Parametric Wilcoxon Rank Sum analysis (Section 22).

Figure 40. Sections 21 and 22 Statistical Analysis [Step 7a of 7] Example 1.

	*** SECTION 21: Overall Conclusion using Parametric Test		
Conclusion	Aquarius supports the rainfall rule change (Parametric)		***
Conclusoin	If samples comply with State Agency sampling guidelines		***
	*** SECTION 22: Overall Conclusion using Non-Parametric Test		
Conclusoin	Aquarius supports the rainfall rule change (Non Parametric)		***
Conclusoin	If samples comply with Sate Agency sampling guidelines		***

Note that both conclusions support the hypothetical New Rule, but contingent on the sampling program employed to have been in compliance with the regulatory agencies' sampling schedule.

This first example was based on a hypothetical new rule proposal that would open more days for shellfish harvest, and based on relaxing the rainfall closure rule. We viewed the total report to illustrate the range of sectional windows that appear, knowing that some statistical runs were not necessary to the analysis. Under this situation, the report stated which sections were applicable to a new rule approval.

Note: An experienced user of the program would have generated the report, switched the filter level to Level 1, if desiring a parametric analysis, or Level 2, if the data was believed to be unevenly distributed and better approached through a non-parametric analysis. The user would then move to the bottom of the report to read the conclusion, and then returned to the top of the report to review each applicable section for a finite analysis of each section.

Example 2. *In example two, we will set up a scenario in which a hypothetical new rule opens up more days for harvest, but one that provides an example of an outcome in which the statistical analysis does not supports the hypothetical new rule. This will allow us to demonstrate additional features of the program, and demonstrate how the analytical results screen displays these negative results.*

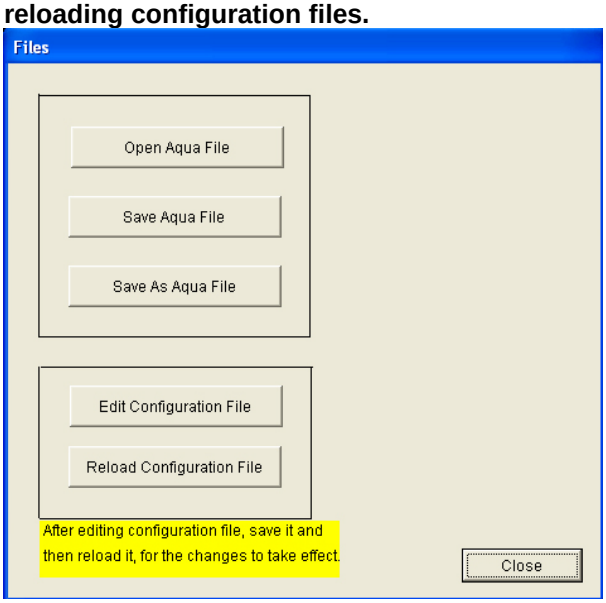
To demonstrate Example 2, the user can either restart the program, or use the [Previous] button to return to Step 3 of 7, which is the first data input screen after the Fecal Coliform database. We will use the same rainfall and fecal coliform databases, but change the parameters for site analysis and rules.

We can also use this occasion to load a previous saved Example 2. This can be done by pressing the "**Previous"** button in Example 1 until the screen Input: Region [Step 3 of 7] is displayed.

Press **Files**

The Files dialog box appears (Figure 41a).

Figure 41a. Files dialog box for opening, saving, saving as, editing configuration and reloading configuration files.



Press Open Aqua Files

Aquarius: Open Aquarius File dialog box appears.

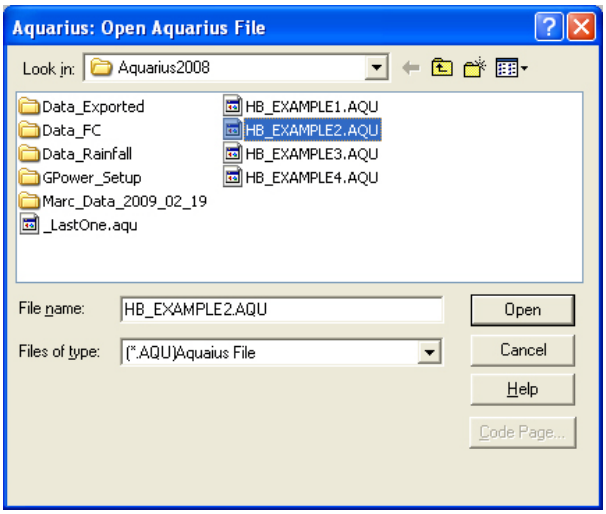


Figure 41b. Aquarius: Open Aquarius File.

Select HB_EXAMPLE2.AQU and press **Open**.

The Input: Region [Step 3 of 7] dialog box appears (Figure 41c). Because the file was loaded from a previous saved scenario, the correct parameters are already entered into the dialog box.

Figure 41c. Input: Region [Step 3 of 7] Example 2.

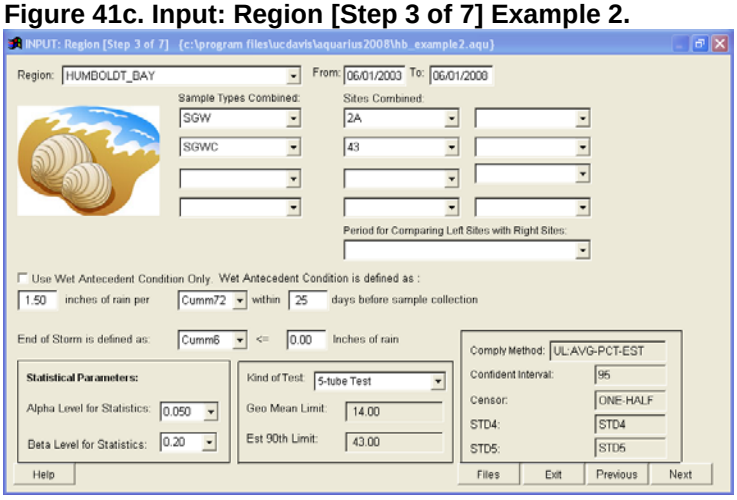


Figure 41c, Input Region, Step 3 of 7 represents the first inputs for Example 2. The region and time range remains the same as HUMBOLDT_BAY, and the entry for June 1, 2003 through June 1, 2008. However in Example 2 we are using two Sample Types Combined (SGW and SGWC) and two Sites Combined (2A and 43). We are not checking Wet Antecedent Conditions, and the statistical parameters and the 5-tube test remain the same as in Example 1. Comply Method = UL:AVG-PCT-EST, Confident Number = 95 and Censor = ONE-HALF.

Press Next to open the Input menu for the Closure Rules.

The dialog box for the Old Closure Rule and the New Closure Rule opens (Figure 42, Input Closure Rules [Step 4 of 7].

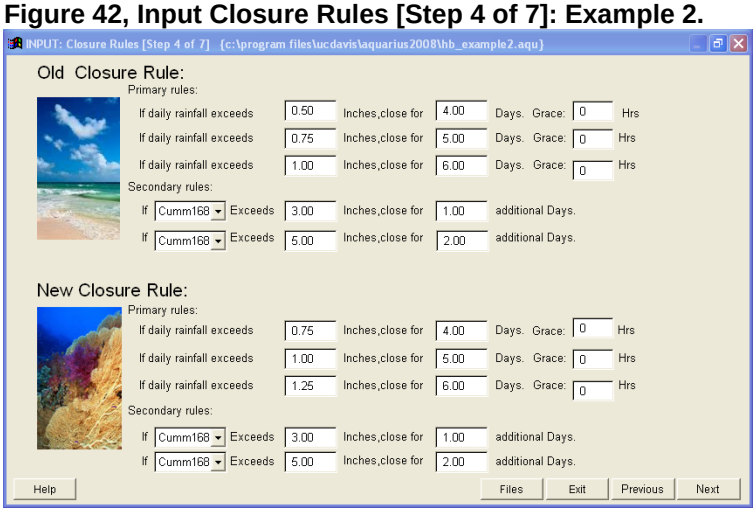
In Figure 42, in the Old Closure Rule, in the Primary Rules, if daily rainfall exceeds 0.5 inches in 24-hours, the sites close for 4-days with no grace period. If the daily rainfall exceeds 0.75-inches, it closes for 5-days, and if it exceeds 1.00-inchs, it is closed for 6-days.

In the Secondary rules, if the 7-day accumulative rainfall exceeds 3-inches, the sites are closed for 1 additional day; and if it exceeds 5-inches, it is closed for 2 additional days.

In the New Rule, in the Primary Rules, the daily rainfall input boxes are changed to 0.75-inches, 1.0-inches and 1.25-inches. All the other criteria remain the same as the Old Rule.

(Review Figure 42 to observe the subtle changes)

Figure 42, Input Closure Rules [Step 4 of 7]: Example 2.



Press **Next**

The dialog box for simulation and statistical analysis opens. **Answer Yes**

The OUTPUTS: Simulated Closures [Step 5 of 7] screen appears.

To speed the process of reading the final analysis, **press Next** until Statistical Analysis [Step 7a of 7] appears.

Set the Filter Level at Filter Level 1 (NSSP and Parametric sections). From Example 1, Filter Level one reveals only the critical sections that are necessary to analyze the results of the NSSP relevant analyses and the parametric t-tests.

Scroll down to SECTON 21 for the conclusion (Figure 43).

Figure 43. Section 21, Statistical Analysis [Step 7a of 7] Conclusion, Example 2.

	*** SECTION 21: Overall Conclusion using Parametric Test		
Conclusion	Aquarius DOES NOT support the rainfall rule change.		***

The conclusion displayed in Figure 43 states that the Aquarius analysis does not statistically support the hypothetical new rule, or rainfall rule change.

With this information, the user can now examine the report to determine why the hypothetical new rule failed. In doing so, you will note that while some sections produce statistical positive results, other sections do not.

Scroll up to Section 2 and examine the statistics for (Old Open), in which the Geometric Mean (3.3) and the Estimated 90th percentile (15.5) are displayed in Figure 44.

Figure 44. Section 2, Statistical Analysis [Step 7a of 7] Example 2.

	*** SECTION 2: Descriptive Statistics for Site Open under Old Rule		
(Old Open)	Count	127	
(Old Open)	Log Average	0.516	
(Old Open)	Log Standard Deviation	0.526	
(Old Open)	Median	2.0	
(Old Open)	Percent > 43	3.1	
(Old Open)	Percent Censored	32.3	
(Old Open)	Geometric Mean	3.3	
(Old Open)	Est 90th Percentile	15.5	
(Old Open)	Geometric Mean (95% Confidence Interval Upper Limit)	4.1	
(Old Open)	Est 90th Percentile (95% Confidence Interval Upper Limit)	19.3	
(Old Open)	>> To meet the standards the Geometric Mean (UL) must be less than 14		
(Old Open)	>> and not more than 10% of the samples shall exceed 43		
(Old Open)	>> and the Est 90th Percentile (UL) must be less than 43 for 5-tube Test.		
(Old Open)	>> This data set meets the above limits.		***
(Old Open)	>> This test supports the rainfall rule change.		***
	*** SECTION 3: Descriptive Statistics for Site Open under New Rule		

In Section 2, the Geometric mean is 3.3 and the Estimated 90th percentile is less than 43 for the 5-tube test, which supports the NSSP fecal coliform standard. The percent greater than 43 is 3.1%, which is less than 10% of the samples. Also note that the GM (95% Confident Interval Upper Limit (CIUL), is 4.1; and the Estimated 90th percentile (95% CIUL) is 19.3. Both of these are more sensitive analytical indicators. The conclusion is that this data set meets the fecal coliform water quality standards and the tests support the rainfall rule change.

Scroll to Section 3 (New Open).

Figure 45. Section 3, Statistical Analysis [Step 7a of 7] Example 2.

	*** SECTION 3: Descriptive Statistics for Site Open under New Rule		
(New Open)	Count	151	
(New Open)	Log Average	0.589	
(New Open)	Log Standard Deviation	0.545	
(New Open)	Median	4.0	
(New Open)	Percent > 43	4.6	
(New Open)	Percent Censored	27.8	
(New Open)	Geometric Mean	3.9	
(New Open)	Est 90th Percentile	19.3	
(New Open)	Geometric Mean (95% Confidence Interval Upper Limit)	4.7	
(New Open)	Est 90th Percentile (95% Confidence Interval Upper Limit)	23.7	
(New Open)	>> To meet the standards the Geometric Mean (UL) must be less than 14		
(New Open)	>> and not more than 10% of the samples shall exceed 43		
(New Open)	>> and the Est 90th Percentile (UL) must be less than 43 for 5-tube Test.		
(New Open)	>> This data set meets the above limits.		***
(New Open)	>> This test supports the rainfall rule change.		***

In Section 3, the Geometric mean is less than 14 and the Estimated 90th percentile is less than 43 for the 5-tube test. As a more sensitive test. GM (95% CIUL) and Est. 90% (95 CIUL) are 4.7 and 23.7, respectively. These results also support the NSSP fecal coliform standard and support the rainfall rule change.

Scroll down to Section 4 (Old Closed, New Open), (Critical Period).

Section 4, which is Old Closed, New Open represents the Critical Period. These are the important samples that were taken when the site was closed under the old rule, but would be open under the new rule. Not only must the samples

comply with the NSSP standards, but the sample size must be adequate and samples taken according to the agency prescribed schedule.

In Figure 46, in Section 4, (Critical Samples), the Geometric Mean 9.4, and the Estimated 90th percentile of 39.4 is less than 43 for the 5-tube test. Both these values comply with the NSSP standards.

Figure 46. Section 4, Critical Period, Statistical Analysis [Step 7a of 7] Example 2.

	*** SECTION 4: Descriptive Statistics for Critical Period A		
(Old Closed, New Open)	Count	24	
(Old Closed, New Open)	Log Average	0.974	
(Old Closed, New Open)	Log Standard Deviation	0.483	
(Old Closed, New Open)	Median	12.0	
(Old Closed, New Open)	Percent > 43	12.5	
(Old Closed, New Open)	Percent Censored	4.2	
(Old Closed, New Open)	Geometric Mean	9.4	
(Old Closed, New Open)	Est 90th Percentile	39.1	
(Old Closed, New Open)	Geometric Mean (95% Confidence Interval Upper Limit)	14.7	
(Old Closed, New Open)	Est 90th Percentile (95% Confidence Interval Upper Limit)	69.4	
(Old Closed, New Open)	>> To meet the standards the Geometric Mean (UL) must be less than 14		
(Old Closed, New Open)	>> and not more than 10% of the samples shall exceed 43		
(Old Closed, New Open)	>> and the Est 90th Percentile (UL) must be less than 43 for 5-tube Test.		
(Old Closed, New Open)	>> This data set DOES NOT meet the above limits.		***
(Old Closed, New Open)	>> This test DOES NOT support the rainfall rule change.		***

However, note that the percent greater than 43 is more than 10% (12.5%) and the high value of the Estimated 90th Percentile (95% CIUL) of 69.4. This latter figure exceeds the limit of less than 43 for a 5-tube test, and Aquarius determines that the data set does not meet the criteria for the above limits, and does not support the new rule change. Section 4, the critical period samples, does not meet the 4th criteria for support of a rule change as seen in Figure 21.

Under these circumstances in which the Estimated 90th Percentile meets the standard, but the Estimated 90th Percentile (95% CIUL) does not, it is usually a result of inadequacy of sample size, and in this case, during the critical sampling period. We will see this in Section 11, but first let's examine Section 10.

Scroll down to Section 10.

Figure 46. Section 10, Statistical Analysis [Step 7a of 7] Example 2.

	*** SECTION 10: Parametric Test Comparing (Critical A) vs (New Closed)		
COMPARISON	(Old Closed, New Open) VS (New Closed)		
T-Test	n1	24	
T-Test	n2	11	
T-Test	Lower t sub a	-2.101	
T-Test	Computed t sub c	0.735	
T-Test	Upper t sub b	2.101	
T-Test	Alpha level	0.050	
T-Test	Degree of freedom	18	
T-Test	>> T-Test IS NOT significant at Alpha = 0.050		
T-Test	>> We can say (Old Closed, New Open) IS EQUAL TO (New Closed)		***
T-Test	>> Required sample size for EACH group is 175.		
T-Test	>> This data set DOES NOT meet the required sample size.		***
T-Test	>> This test DOES NOT support the rainfall rule change.		***
T-Test	The following values are useful for calculating required sample size:		
T-Test	n1= 24, Avg1= 0.974, Std1= 0.483, n2= 11, Avg2= 0.825, Std2= 0.589		
T-Test	Pooled Std= 0.502, Diff Avg = 0.149, Effect Size d = 0.300		

Figure 46 displays T-test results from a comparison of Old Closed, New Open (n1) against New Closed (n2). The results show that the T-test is not significant at Alpha = 0.05 and that (Old Closed, New Open) is equal to (New Closed). Also, that the required sample size for each group (n1 and n2) is 175. In our scenario, n1 = 24 samples and n2 = 11 samples, which is insufficient, and this is confirmed by the Sample Size statistical program. In the Sample Size program, be sure Section 10 is indicated and Effect size d = 0.3. (as displayed in the last line of the T-test. This test as displayed in Section 10 does not support the new rule change.

Scroll down to Section 11 and Section 21, the Conclusions.

Figure 47. Section 11 and Section 21, Conclusions Statistical Analysis [Step 7a of 7] Example 2.

	*** SECTION 11: Parametric Test Comparing (Critical A) vs (Old Open)		
COMPARISON	(Old Closed, New Open) VS (Old Open)		
T-Test	n1	24	
T-Test	n2	127	
T-Test	Lower t sub a	-2.030	
T-Test	Computed t sub c	4.195	
T-Test	Upper t sub b	2.030	
T-Test	Alpha level	0.050	
T-Test	Degree of freedom	35	
T-Test	>> T-Test IS significant at Alpha = 0.050		
T-Test	>> (Old Closed, New Open) IS GREATER THAN (Old Open)		***
T-Test	>> Required sample size for EACH group is 20.		
T-Test	>> This data set meets the required sample size.		***
T-Test	>> This test supports the rainfall rule change.		***
T-Test	The following values are useful for calculating required sample size:		
T-Test	n1= 24, Avg1= 0.974, Std1= 0.483, n2= 127, Avg2= 0.516, Std2= 0.526		
T-Test	Pooled Std= 0.516, Diff Avg = 0.458, Effect Size d = 0.886		
	*** SECTION 21: Overall Conclusion using Parametric Test		
Conclusion	Aquarius DOES NOT support the rainfall rule change.		***

Figure 47 displays Section 11 in which Old Closed, New Open (n1) is compared to Old Open (n2). The results show that the T-test is significant at Alpha = 0.05 and that (Old Closed, New Open) is greater than (New Closed). Although Old Closed, New Open is grater than Old Open, and meets criteria 6 in Figure 21, sample size must also be adequate. The required sample size for each group (n1

and n2) is 20. In our scenario, n1 = 24 samples and n2 = 127 samples, and this is confirmed by the Sample Size statistical program. The sample size is sufficient to support the test. This test displayed in Section 10 supports the new rainfall closure rule.

However, the conclusion stated in Section 21 states that Aquarius **Does Not support the new rule**. The hypothetical new rule is not supported because there are insufficient samples taken during the Critical Period (Section 4). The critical period samples do not meet the 4th criteria for approval of a new rule as displayed in Figure 21.

Example 3: *In example three, we will set up a scenario in which a hypothetical new rule is more restrictive, resulting in less days open for harvest. In addition, this example is used to demonstrate an outcome in which the statistical analysis supports the more restrictive hypothetical rule.*

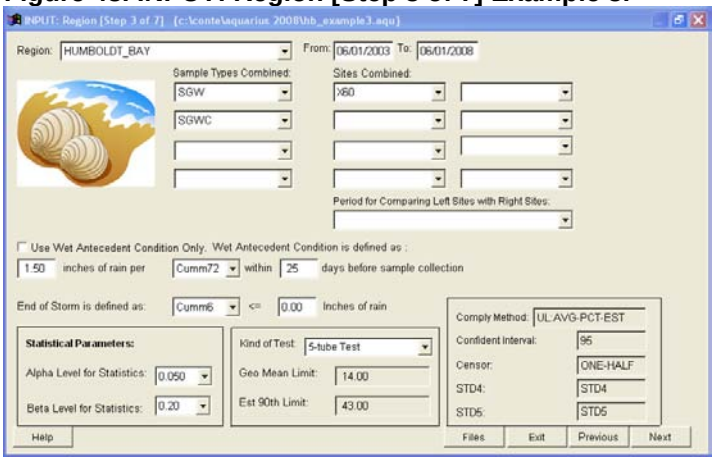
Press the Previous button until INPUT: Region [Step 3 of 7] appears.

Press **Files**, and open **Aqua Files**.

Select **HB_EXAMPLE3.AQU** and press **Open**.

The INPUT: Region [Step 3 of 7] screen appears with the preloaded dialog boxes (Figure 48).

Figure 48. INPUT: Region [Step 3 of 7] Example 3.



In Figure 48, all the input parameters remain the same as that of Example 2, except for the single Site Combined entry of X60.

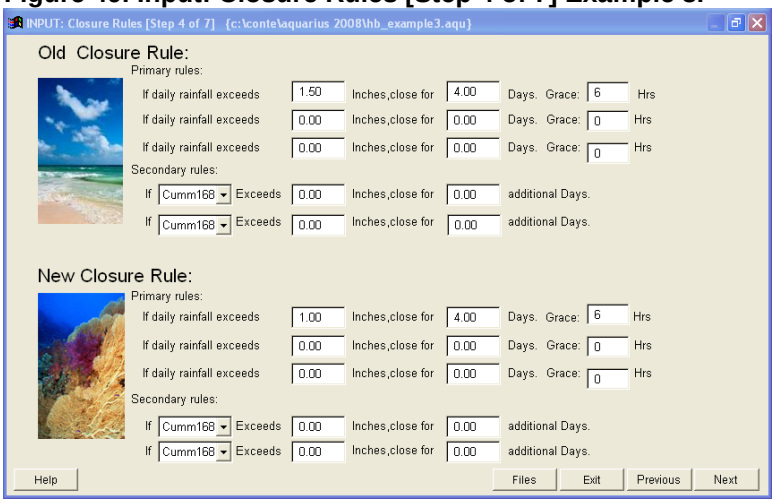
Press **Next**.

The Input: Closure Rules [Step 4 of 7] screen appears (Figure 49).

The Primary Rules of the Old Closure Rule reads, if the daily rainfall exceeds 1.5-inches, close for 4-days after a grace period of 6 hours. This is a simple application rule.

The Primary Rule of the New Closure Rule reads, if the daily rainfall exceeds 1.0-inchs, close the site for 4-days after the 6-hour grace period. This is an example of a change in the rule that is more restrictive and closes the site for more days.

Figure 49. Input: Closure Rules [Step 4 of 7] Example 3.



At this point, you may want to review Figure 22, which is a diagrammatic representation of fecal coliform sampling during the Critical Period in a scenario that would make the rainfall closure rule more restrictive. There are 6 criteria that must be met to approve a more restrictive new rule:

1. The Sample size must be adequate (Section 1)
2. Old Open must violate NSSP (Section 2)
3. New Open must comply with NSSP (Section 3)
4. The Critical Period must violate NSSP (Section 5)
5. Critical Period must be more than New Open (Sections 10 or 14)
6. Critical Period can be less than or equal to Old Closed (Sections 11 or 15)

Press **Next** in Step 4 of 7.

Follow the instructions to run the analysis.

You can review the resultant screens at different filter levels, but to achieve a quick analysis press **Next** until Step 7b of 7 appears.

Set the screen to Filter Level 1 to reveal Sections 2, 3, 5, 10, 11, and 21.

Scroll down to reveal the overall conclusion in Section 21 (Figure 50).

Figure 50. Section 21, Conclusion, using Parametric Test, Example 3.

	*** SECTION 21: Overall Conclusion using Parametric Test		
Conclusion	Aquarius supports the rainfall rule change (Parametric)		***
Conclusoin	If samples comply with State Agency sampling guidelines		***

Figure 50 displays Section 21, which states that Aquarius supports the new rule change in a parametric analysis, if the samples comply with State Agency guidelines.

For a detailed analysis, scroll back up to Section 2.

Figure 51 displays Section 2, which shows the descriptive statistics for Site Open under the Old Rule.

Figure 51. Section 2: Descriptive statistics for site open under the Old Rule, Example 3.

	*** SECTION 2: Descriptive Statistics for Site Open under Old Rule		
(Old Open)	Count	194	
(Old Open)	Log Average	0.942	
(Old Open)	Log Standard Deviation	0.629	
(Old Open)	Median	8.0	
(Old Open)	Percent > 43	16.5	
(Old Open)	Percent Censored	0.0	
(Old Open)	Geometric Mean	8.8	
(Old Open)	Est 90th Percentile	55.8	
(Old Open)	Geometric Mean (95% Confidence Interval Upper Limit)	10.7	
(Old Open)	Est 90th Percentile (95% Confidence Interval Upper Limit)	68.6	
(Old Open)	>> To meet the standards the Geometric Mean (UL) must be less than 14		
(Old Open)	>> and not more than 10% of the samples shall exceed 43		
(Old Open)	>> and the Est 90th Percentile (UL) must be less than 43 for 5-tube Test.		
(Old Open)	>> This data set DOES NOT meet the above limits.		***
(Old Open)	>> This test supports the rainfall rule change.		***

In Figure 51, the critical parameters include:

Percent greater than 43 = 16.5 percent (not more than 10 % should be >43)

Geometric Mean = 8.8; Estimated 90th percentile = 55.8

Geometric Mean Upper Limit = 10.7; Estimated 90th percentile Upper Limit = 68.6

The data set does not meet the fecal coliform water quality standards; however, this test supports the new more restrictive rule, as it meets the second requirement of Figure 22, in that Old Open **must** violate NSSP.

Scroll down to Section 3.

Figure 52. Section 3: Descriptive statistics for site open under the New Rule, Example 3.

	*** SECTION 3: Descriptive Statistics for Site Open under New Rule		
(New Open)	Count	120	
(New Open)	Log Average	0.586	
(New Open)	Log Standard Deviation	0.395	
(New Open)	Median	2.0	
(New Open)	Percent > 43	3.3	
(New Open)	Percent Censored	0.0	
(New Open)	Geometric Mean	3.9	
(New Open)	Est 90th Percentile	12.3	
(New Open)	Geometric Mean (95% Confidence Interval Upper Limit)	4.5	
(New Open)	Est 90th Percentile (95% Confidence Interval Upper Limit)	14.6	
(New Open)	>> To meet the standards the Geometric Mean (UL) must be less than 14		
(New Open)	>> and not more than 10% of the samples shall exceed 43		
(New Open)	>> and the Est 90th Percentile (UL) must be less than 43 for 5-tube Test.		
(New Open)	>> This data set meets the above limits.		***
(New Open)	>> This test supports the rainfall rule change.		***

In Figure 52, the critical parameters include:

Percent greater than 43 = 3.3 percent (not more than 10 % should be >43)

Geometric Mean = 3.9; Estimated 90th percentile = 12.3

Geometric Mean Upper Limit = 4.5; Estimated 90th percentile Upper Limit = 14.6

The data set meets the fecal coliform water quality standards, and this test supports the new rule change, as New Open must comply with NSSP.

Scroll down to Section 5.

Figure 53. Section 5, Descriptive statistics for Critical Period B, Example 3.

	*** SECTION 5: Descriptive Statistics for Critical Period B		
(Old Open, New Closed)	Count	74	
(Old Open, New Closed)	Log Average	1.519	
(Old Open, New Closed)	Log Standard Deviation	0.495	
(Old Open, New Closed)	Median	24.0	
(Old Open, New Closed)	Percent > 43	37.8	
(Old Open, New Closed)	Percent Censored	0.0	
(Old Open, New Closed)	Geometric Mean	33.1	
(Old Open, New Closed)	Est 90th Percentile	142.4	
(Old Open, New Closed)	Geometric Mean (95% Confidence Interval Upper Limit)	42.9	
(Old Open, New Closed)	Est 90th Percentile (95% Confidence Interval Upper Limit)	188.8	
(Old Open, New Closed)	>> To meet the standards the Geometric Mean (UL) must be less than 14		
(Old Open, New Closed)	>> and not more than 10% of the samples shall exceed 43		
(Old Open, New Closed)	>> and the Est 90th Percentile (UL) must be less than 43 for 5-tube Test.		
(Old Open, New Closed)	>> This data set DOES NOT meet the above limits.		***
(Old Open, New Closed)	>> This test supports the rainfall rule change.		***

Figure 53 addresses the Critical Period B samples, and the critical parameters include:

Percent greater than 43 = 37.8 percent (not more than 10 % should be >43)

Geometric Mean = 33.1; Estimated 90th percentile = 142.4

Geometric Mean Upper Limit = 42.9; Estimated 90th percentile Upper Limit = 188.8

The data set does not meet the fecal coliform water quality standards. However, this test supports the Critical Period (Old Open, New Closed), as the **Critical Period must violate NSSP when the new rule is more restrictive**.

Scroll down to Section 10.

Figure 54, Section 10 displays the parametric test comparing (Critical B) with (New Open).

Figure 54, Section 10 displaying the parametric test comparing (Critical B) with (New Open), Example 3.

	*** SECTION 10: Parametric Test Comparing (Critical B) vs (New Open)		
COMPARISON	(Old Open, New Closed) VS (New Open)		
T-Test	n1	74	
T-Test	n2	120	
T-Test	Lower t sub a	-1.978	
T-Test	Computed t sub c	13.736	
T-Test	Upper t sub b	1.978	
T-Test	Alpha level	0.050	
T-Test	Degree of freedom	131	
T-Test	>> T-Test IS significant at Alpha = 0.050		
T-Test	>> (Old Open, New Closed) IS GREATER THAN (New Open)		***
T-Test	>> Required sample size for EACH group is 16.		
T-Test	>> This data set meets the required sample size.		***
T-Test	>> This test supports the rainfall rule change.		***
T-Test	The following values are useful for calculating required sample size:		
T-Test	n1= 74, Avg1= 1.519, Std1= 0.495, n2= 120, Avg2= 0.586, Std2= 0.395		
T-Test	Pooled Std= 0.433, Diff Avg = 0.933, Effect Size d = 1.000		

In Figure 54, the critical parameters are:

n1 = 74

n2 = 120

The T-test is significant at Alpha = 0.05

(Old Open, New Closed) is greater than (New Open)

Required sample size for each group is 16, and the data meets this requirement.

This test supports the New Rule, as the Critical Period must be more than New Open.

Scroll down to Section 11.

Figure 55 displays Sections 11, the parametric T-test comparing (critical B) and (Old Closed) and Section 21 the overall conclusion using the parametric test.

In Figure 55, the critical parameters are:

n1 = 74

n2 = 179

The T-test is significant at Alpha = 0.05

(Old Open, New Closed) is less than (Old Closed)

Required sample size for each group is 16, and the data meets this requirement.

This test supports the New Rule, as the Critical Period must be more than New Open.

Figure 55 displaying Sections 11, the parametric T-test comparing (critical B) and (Old Closed) and Section 21 the overall conclusion using the parametric test.

	*** SECTION 11: Parametric Test Comparing (Critical B) vs (Old Closed)		
COMPARISON	(Old Open, New Closed) VS (Old Closed)		
T-Test	n1	74	
T-Test	n2	179	
T-Test	Lower t sub a	-1.977	
T-Test	Computed t sub c	-8.654	
T-Test	Upper t sub b	1.977	
T-Test	Alpha level	0.050	
T-Test	Degree of freedom	142	
T-Test	>> T-Test IS significant at Alpha = 0.050		
T-Test	>> (Old Open, New Closed) IS LESS THAN (Old Closed)		***
T-Test	>> Required sample size for EACH group is 16.		
T-Test	>> This data set meets the required sample size.		***
T-Test	>> This test supports the rainfall rule change.		***
T-Test	The following values are useful for calculating required sample size:		
T-Test	n1= 74, Avg1= 1.519, Std1= 0.495, n2= 179, Avg2= 2.117, Std2= 0.510		
T-Test	Pooled Std= 0.504, Diff Avg = 0.598, Effect Size d = 1.000		
	*** SECTION 21: Overall Conclusion using Parametric Test		
Conclusion	Aquarius supports the rainfall rule change (Parametric)		***
Conclusoin	If samples comply with State Agency sampling guidelines		***

As stated earlier, Section 21 states that Aquarius supports the new, more restrictive rule. The sample sizes are adequate for the analysis and the criteria stated in Figure 22 for approving a more restrictive rule are met.

Try using the Sample Size program. Also, go to Appendix F and examine the GPower program. GPower is another more powerful sample size program to check the sample size statistics.

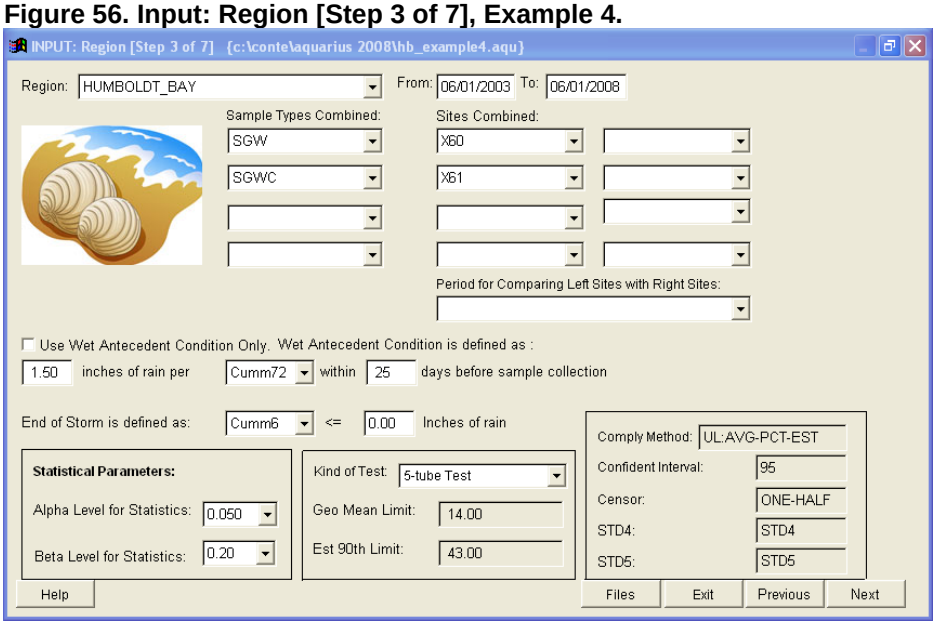
Example 4: ***In example four, we will set up a scenario in which a hypothetical new rule is more restrictive, resulting in less days open for harvest. In addition, this example is used to demonstrate an outcome in which the statistical analysis does not support the more restrictive hypothetical rule.***

Press the **Previous** button until the Input: Region [Step 3 of 7] screen appears.

Use the **Files** button to open the Files menu, **select Open Aqua File, select HB_EXAMPLE4.AQU**, and **Press Open**

The screen, Input: Region [Step 3 of 7] appears (Figure 56).

Figure 56. Input: Region [Step 3 of 7], Example 4.

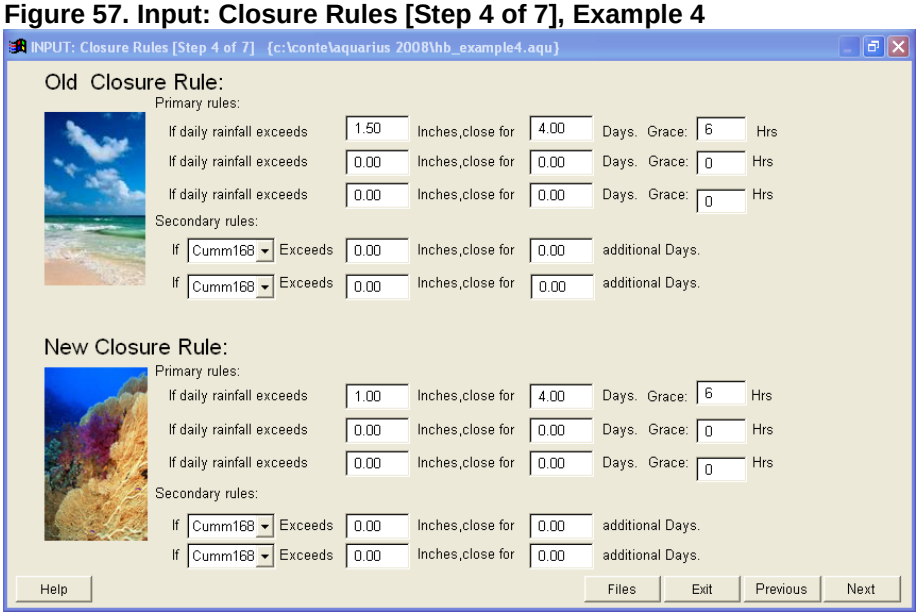


In Figure 56, all the input parameters remain the same as that of Example 3, except for the addition of site X61 to site X60.

Press **Next**.

The Input: Closure Rules [Step 4 of 7] screen appears (Figure 57).

Figure 57. Input: Closure Rules [Step 4 of 7], Example 4



The Primary Rules of the Old Closure Rule reads, if the daily rainfall exceeds 1.5-inches, close for 4-days after a grace period of 6 hours. This is a simple application rule just like in the previous example.

The Primary Rule of the New Closure Rule reads, if the daily rainfall exceeds 1.0-inchs, close the site for 4-days after the 6-hour grace period. This is an example of a change in the rule that is more restrictive and closes the site for more days.

Note: The only difference in Example 4 from Example 3 was the addition of an additional site, X61.

Press Next, to run the simulation and statistical analysis.

Press Next, to reveal the output screens. Continue to **Press Next**, until the final report screen Step 7b of 7 is displayed.

Set the screen to Filter Level 1 to reveal Sections 2, 3, 5, 10, 11, and 21.

Scroll down to reveal the overall conclusion in Section 21 (Figure 58).

Figure 58. Section 21, Overall Conclusion using Parametric Test Example 4.

	*** SECTION 21: Overall Conclusion using Parametric Test		
Conclusion	Aquarius DOES NOT support the rainfall rule change.		***

Figure 58 displays Section 21, which states that Aquarius does not support the new rule in a parametric analysis.

For a detailed analysis, scroll back up to Section 2.

Figure 59. Section 2, Descriptive analysis for site open under New Rule, Example 4

	*** SECTION 2: Descriptive Statistics for Site Open under Old Rule		
(Old Open)	Count	394	
(Old Open)	Log Average	0.690	
(Old Open)	Log Standard Deviation	0.550	
(Old Open)	Median	2.0	
(Old Open)	Percent > 43	8.1	
(Old Open)	Percent Censored	0.0	
(Old Open)	Geometric Mean	4.9	
(Old Open)	Est 90th Percentile	24.8	
(Old Open)	Geometric Mean (95% Confidence Interval Upper Limit)	5.6	
(Old Open)	Est 90th Percentile (95% Confidence Interval Upper Limit)	26.4	
(Old Open)	>> To meet the standards the Geometric Mean (UL) must be less than 14		
(Old Open)	>> and not more than 10% of the samples shall exceed 43		
(Old Open)	>> and the Est 90th Percentile (UL) must be less than 43 for 5-tube Test.		
(Old Open)	>> This data set meets the above limits.		***
(Old Open)	>> This test DOES NOT support the rainfall rule change.		***

In Figure 59, the critical parameters include:

Percent greater than 43 = 8.1 percent (not more than 10 % should be >43)

Geometric Mean = 4.9; Estimated 90th percentile = 24.8

Geometric Mean Upper Limit = 5.6; Estimated 90th percentile Upper Limit = 36.8

The data set meets the fecal coliform water quality standards, but this data set does not support the new rule, as **it does not meet the second requirement of Figure 22, that Old Open must violate NSSP when the new rule is more restrictive**.

Scroll down to Section 3.

Figure 60, displays the descriptive statistics for site open under the New Rule.

Figure 60. Section 3, descriptive statistics for site open under New Rule, Example 4.

	*** SECTION 3: Descriptive Statistics for Site Open under New Rule		
(New Open)	Count	320	
(New Open)	Log Average	0.499	
(New Open)	Log Standard Deviation	0.346	
(New Open)	Median	2.0	
(New Open)	Percent > 43	1.3	
(New Open)	Percent Censored	0.0	
(New Open)	Geometric Mean	3.2	
(New Open)	Est 90th Percentile	8.8	
(New Open)	Geometric Mean (95% Confidence Interval Upper Limit)	3.4	
(New Open)	Est 90th Percentile (95% Confidence Interval Upper Limit)	9.3	
(New Open)	>> To meet the standards the Geometric Mean (UL) must be less than 14		
(New Open)	>> and not more than 10% of the samples shall exceed 43		
(New Open)	>> and the Est 90th Percentile (UL) must be less than 43 for 5-tube Test.		
(New Open)	>> This data set meets the above limits.		***
(New Open)	>> This test supports the rainfall rule change.		***

In Figure 60, the critical parameters include:

Percent greater than 43 = 1.3 percent (not more than 10 % should be >43)

Geometric Mean = 3.2; Estimated 90th percentile = 8.8

Geometric Mean Upper Limit = 3.4; Estimated 90th percentile Upper Limit = 9.9

The data set meets the fecal coliform water quality standards, and this data test meets third requirement of Figure 22, that New Open must comply with NSSP.

Scroll down to Section 5.

Figure 61, displays Section 5, Descriptive statistics for Critical Period B.

Figure 61, Section 5, Descriptive statistics for Critical Period B. Example 4.

	*** SECTION 5: Descriptive Statistics for Critical Period B		
(Old Open, New Closed)	Count	74	
(Old Open, New Closed)	Log Average	1.519	
(Old Open, New Closed)	Log Standard Deviation	0.495	
(Old Open, New Closed)	Median	24.0	
(Old Open, New Closed)	Percent > 43	37.8	
(Old Open, New Closed)	Percent Censored	0.0	
(Old Open, New Closed)	Geometric Mean	33.1	
(Old Open, New Closed)	Est 90th Percentile	142.4	
(Old Open, New Closed)	Geometric Mean (95% Confidence Interval Upper Limit)	42.9	
(Old Open, New Closed)	Est 90th Percentile (95% Confidence Interval Upper Limit)	188.8	
(Old Open, New Closed)	>> To meet the standards the Geometric Mean (UL) must be less than 14		
(Old Open, New Closed)	>> and not more than 10% of the samples shall exceed 43		
(Old Open, New Closed)	>> and the Est 90th Percentile (UL) must be less than 43 for 5-tube Test.		
(Old Open, New Closed)	>> This data set DOES NOT meet the above limits.		***
(Old Open, New Closed)	>> This test supports the rainfall rule change.		***

In Figure 61, the critical parameters include:

Percent greater than 43 = 37.8 percent (not more than 10 % should be >43)

Geometric Mean = 33.1; Estimated 90th percentile = 142.4

Geometric Mean Upper Limit = 42.9; Estimated 90th percentile Upper Limit = 188.8

The data set does not meet the fecal coliform water quality standards, but this data test supports the new rule, as it meets fourth requirement of Figure 22, in that **the Critical Period must violate NSSP if the new rule is more restrictive**.

Scroll down to Section 10.

Figure 62, displays Section 10, parametric test comparing (Critical B) with (Old Open).

Figure 62, displaying Section 10, parametric test comparing (Critical B) with (Old Open), Example 4.

	*** SECTION 10: Parametric Test Comparing (Critical B) vs (New Open)		
COMPARISON	(Old Open, New Closed) VS (New Open)		
T-Test	n1	74	
T-Test	n2	320	
T-Test	Lower t sub a	-1.986	
T-Test	Computed t sub c	16.797	
T-Test	Upper t sub b	1.986	
T-Test	Alpha level	0.050	
T-Test	Degree of freedom	91	
T-Test	>> T-Test IS significant at Alpha = 0.050		
T-Test	>> (Old Open, New Closed) IS GREATER THAN (New Open)		***
T-Test	>> Required sample size for EACH group is 16.		
T-Test	>> This data set meets the required sample size.		***
T-Test	>> This test supports the rainfall rule change.		***
T-Test	The following values are useful for calculating required sample size:		
T-Test	n1= 74, Avg1= 1.519, Std1= 0.495, n2= 320, Avg2= 0.499, Std2= 0.346		
T-Test	Pooled Std= 0.378, Diff Avg = 1.021, Effect Size d = 1.000		

In Figure 62, the critical parameters include:

n1 = 74

n2 = 320

The T-test is significant at Alpha = 0.05

(Old Open, New Closed) is greater than (New Open)

Required sample size for each group is 16, and the data meets this requirement.

<u>This test supports the New Rule, as the Critical Period must be greater than New Open</u>.

Scroll down to Sections 11 and 21.

Figure 63 displays Section 11, which is a parametric test comparing (Critical B) with (Old Closed); and Section 21, the overall conclusion using the parametric test.

Figure 63. Displaying Section 11, a parametric test comparing (Critical B) with (Old Closed); and Section 21, the overall conclusion using the parametric test, Example 4.

	*** SECTION 11: Parametric Test Comparing (Critical B) vs (Old Closed)		
COMPARISON	(Old Open, New Closed) VS (Old Closed)		
T-Test	n1	74	
T-Test	n2	179	
T-Test	Lower t sub a	-1.977	
T-Test	Computed t sub c	-8.654	
T-Test	Upper t sub b	1.977	
T-Test	Alpha level	0.050	
T-Test	Degree of freedom	142	
T-Test	>> T-Test IS significant at Alpha = 0.050		
T-Test	>> (Old Open, New Closed) IS LESS THAN (Old Closed)		***
T-Test	>> Required sample size for EACH group is 16.		
T-Test	>> This data set meets the required sample size.		***
T-Test	>> This test supports the rainfall rule change.		***
T-Test	The following values are useful for calculating required sample size:		
T-Test	n1= 74, Avg1= 1.519, Std1= 0.495, n2= 179, Avg2= 2.117, Std2= 0.510		
T-Test	Pooled Std= 0.504, Diff Avg = 0.598, Effect Size d = 1.000		
	*** SECTION 21: Overall Conclusion using Parametric Test		
Conclusion	Aquarius DOES NOT support the rainfall rule change.		***

In Figure 63, the critical parameters include:

n1 = 74

n2 = 179

The T-test is significant at Alpha = 0.05

(Old Open, New Closed) is less than (New Open)

Required sample size for each group is 16, and the data meets this requirement.

The test in Section 11 supports the New Rule, as the Critical Period can be less than or equal to Old Closed, which meets the 6th criteria for approving the new rule. However, the overall test (seen in the conclusion of Section 21, does not support the new rule because Section 2 did not meet the 2nd criteria as seen in Figure 22.

Example 5: ***In example five, we will look at another type of analysis. In this analysis, we are not interested in analyzing a new hypothetical rule, but we are interested in examining two data sets to determine is there significant differences between the data sets. This type of analysis can be used to determine if sites, or combination of sites, might be combined. In this example, we will examine the combined sites of 22 and 24 to determine if there is a significant difference between these sites and site 51, which is located in the same bay.***

Press the **Previous button** until INPUT: Region [Step 3 of 7] appears.

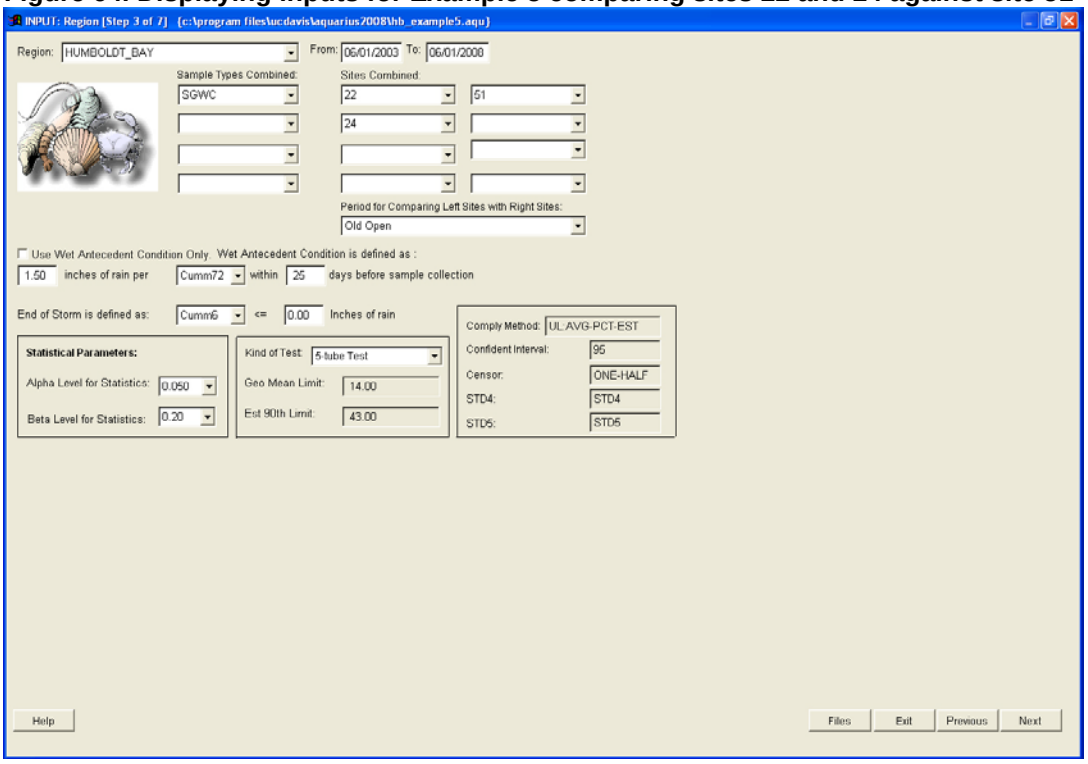
Press **Files** to open the Files dialog box.

Press **Open Aqua Files** to reveal the HP_EXAMPLE files.

Select HB_EXAMPLE5.AQU and press **Open**.

Step 3 of 7 appears with the information preset for Example 5 (Figure 64).

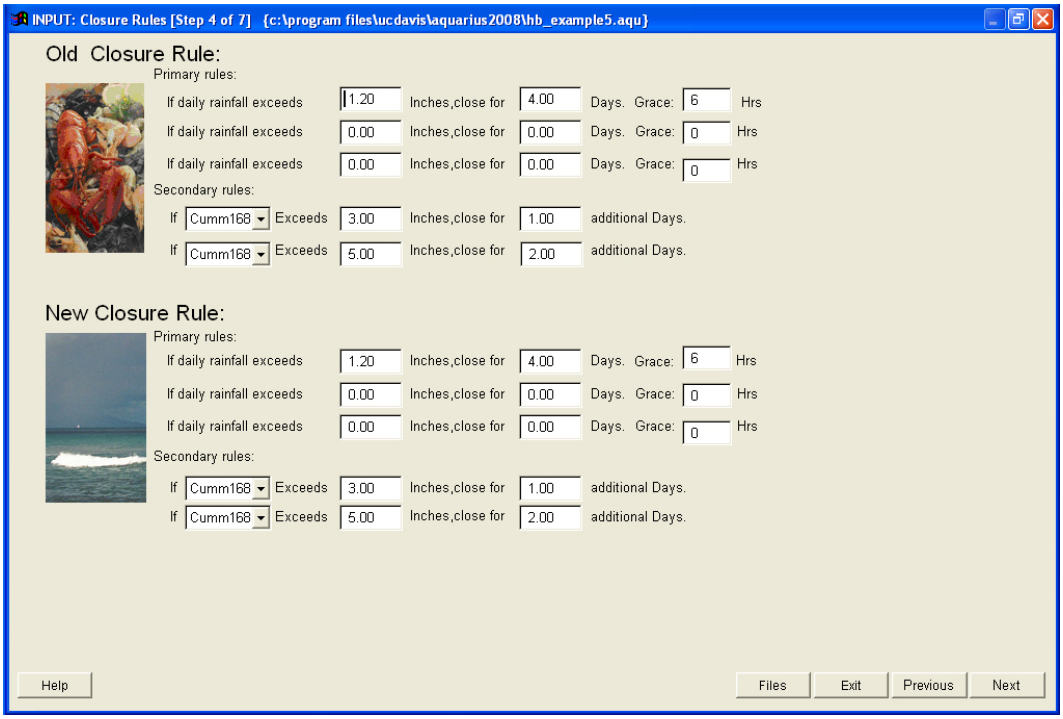
Figure 64. Displaying inputs for Example 5 comparing sites 22 and 24 against site 51



Press **Next**.

INPUTS: Closure Rules [Step 4 of 7] appears (Figure 65).

Figure 65. Displaying parameters for Example 5, in which there are no differences listed for a new closure rule.



Because we are only determining if there are significant differences between sites 22 and 24 when compared to site 51, the parameters listed in the Old Closure Rule are repeated in the options for the New Closure Rule. The input data should be identical.

Press **Next**.

You are asked if you want to run simulation routines and statistical analysis. Press **Yes**.

After the data sheet is exhibited, press next until you reach the final screen and set the Filter Level at 4.

Examine Section 16: Site Information (Figure 66).

Figure 66. Section 16, Example 5 site information.

Category	Description	Value	Star
	*** SECTION 16: Site Information		
Left Sites	22 24		
Right Sites	51		
Period	(Old Open)		

Section 16 displays the Left Sites (22 and 24) and the Right Sites (51) for the Period, Old Open. We are only interested in the open periods in the selected data sets.

Scroll to Section 17 (Figure 67).

Figure 67. Section 17, Descriptive statistics for (Left Sites).

Category	Description	Value	Star
	*** SECTION 17: Descriptive Statistics for (Left Sites)		
(Left Sites)	Count	94	
(Left Sites)	Log Average	0.232	
(Left Sites)	Log Standard Deviation	0.407	
(Left Sites)	Median	0.9	
(Left Sites)	Percent > 43	0.0	
(Left Sites)	Percent Censored	59.6	
(Left Sites)	Geometric Mean	1.7	
(Left Sites)	Est 90th Percentile	5.7	
(Left Sites)	Geometric Mean (95% Confidence Interval Upper Limit)	2.1	
(Left Sites)	Est 90th Percentile (95% Confidence Interval Upper Limit)	6.9	
(Left Sites)	>> To meet the standards the Geometric Mean must be less than 14		
(Left Sites)	>> and not more than 10% of the samples shall exceed 43		
(Left Sites)	>> and the Est 90th must be less than 43 for 5-tube Test.		
(Left Sites)	>> This data set meets the above limits.		***

In Figure 67, the critical parameters include:

Percent greater than 43 = 0.0 percent (not more than 10 % should be >43)

Geometric Mean = 1.7; Estimated 90th percentile = 5.7

Geometric Mean Upper Limit = 2.1; Estimated 90th percentile Upper Limit = 6.9

This data set meets the above limits.

Scroll to Section 18 (Figure 68).

Figure 18. Section 17, Descriptive statistics for (Right Sites).

	*** SECTION 18: Descriptive Statistics for (Right Sites)		
(Right Sites)	Count	47	
(Right Sites)	Log Average	0.208	
(Right Sites)	Log Standard Deviation	0.432	
(Right Sites)	Median	0.9	
(Right Sites)	Percent > 43	0.0	
(Right Sites)	Percent Censored	66.0	
(Right Sites)	Geometric Mean	1.6	
(Right Sites)	Est 90th Percentile	5.8	
(Right Sites)	Geometric Mean (95% Confidence Interval Upper Limit)	2.1	
(Right Sites)	Est 90th Percentile (95% Confidence Interval Upper Limit)	8.0	
(Right Sites)	>> To meet the standards the Geometric Mean must be less than 14		
(Right Sites)	>> and not more than 10% of the samples shall exceed 43		
(Right Sites)	>> and the Est 90th must be less than 43 for 5-tube Test.		
(Right Sites)	>> This data set meets the above limits.		***

In Figure 68, the critical parameters include:

Percent greater than 43 = 0.0 percent (not more than 10 % should be >43)

Geometric Mean = 1.6; Estimated 90th percentile = 5.8

Geometric Mean Upper Limit = 2.1; Estimated 90th percentile Upper Limit = 8.0

This data set meets the above limits.

Scroll to Section 19 (Figure 69).

Figure 69. Section 19, Parametric test comparing (Left Sites) vs. (Right Sites).

	*** SECTION 19: Parametric Test Comparing (Left Sites) vs (Right Sites)		
COMPARISON	(Left Sites) VS (Right Sites)		
T-Test	n1	94	
T-Test	n2	47	
T-Test	Lower t sub a	-1.987	
T-Test	Computed t sub c	0.312	
T-Test	Upper t sub b	1.987	
T-Test	Alpha level	0.050	
T-Test	Degree of freedom	89	
T-Test	>> T-Test IS NOT significant at Alpha = 0.050		
T-Test	>> We can say (Left Sites) IS EQUAL TO (Right Sites)		***
T-Test	The following values are useful for calculating required sample size:		
T-Test	n1= 94, Avg1= 0.232, Std1= 0.407, n2= 47, Avg2= 0.208, Std2= 0.432		
T-Test	Pooled Std= 0.413, Diff Avg = 0.024, Effect Size d = 0.300		

In Figure 69, the critical parameters include:

n1 = 94

n2 = 47

The T-test is Not significant at Alpha = 0.05

Therefore, we can say that there is no significant difference between the two data sets and that the Left Sites is equal to the Right Sites.

Scroll to Section 20 (Figure 70).

Figure 70. Section 20, Non-Parametric test comparing Left Sites) vs. (Right Sites).

	*** SECTION 20: Non-Parametric Test Comparing (Left Sites) vs (Right Sites)		
COMPARISON	(Left Sites) VS (Right Sites)		
NonParam	Nx	94	
NonParam	Ny	47	
NonParam	Rank Sum Rx	6809.000	
NonParam	Rank Sum Ry	3202.000	
NonParam	Computed Z	0.590	
NonParam	Two-sided Z	1.960	
NonParam	P value	0.555	
NonParam	>> Wilcoxon Rank Sum Test IS NOT significant at Alpha = 0.050		
NonParam	>> We can say (Left Sites) IS EQUAL TO (Right Sites)		***

Figure 70 displays a Wilcoxon Rank Sum (WRS) test results from a comparison Left Sites (Nx) against Right Sites (Ny).

The results show that the WRS is significant at Alpha = 0.05 and that (Left Sites) is equal to (Right Sites).

Appendix A.

How to install Aquarius 2.0.

1. Insert the AQUARIUS CD into the CD-Rom drive.

2. Using your Windows Explore, navigate to the CD and you will see a file called Setup.exe.

3. Double click Setup.exe. The Welcome dialog box appears. Click the Next button.

4. The License Agreement dialog box appears. Accept the terms in the license agreement and click the Next button.

5. The Customer Information dialog box appears. Enter your name and your organization and click the Next button.

6. The “Ready to Install the Program” dialog box appears. Click the Install button.

7. The wizard will install the AQUARIUS program on your computer. It may take SEVERAL minutes.

8. Finally, the final dialog box appears. Click the Finish button. The wizard finishes the installation and will place a shortcut to AQUARIUS 2008 on your desktop

9. It will create a folder called:

   “C:\Program Files\UCDAVIS\AQUARIUS2008\” and will place the **AQUARIUS.exe** file in that folder

AQUARIUS Manual

The AQUARIUS manual, **Manual.pdf**, is stored in the AQUARIUS folder. To view and print this file, you need Adobe Acrobat Reader. You can get a free copy of this software by visiting the following web site:

http://www.adobe.com/products/acrobat/readstep2.html

How to Run Aquarius 2.0

1. Click the Start button and then choose the “All Programs”. A menu list appears.

2. Choose the “UCDAVIS” option. A sub-menu appears.

3. Choose the “AQUARIUS2008” option. Another sub-menu appears. Choose the “Launch AQUARIUS” option. The main menu of AQUARIUS appears.

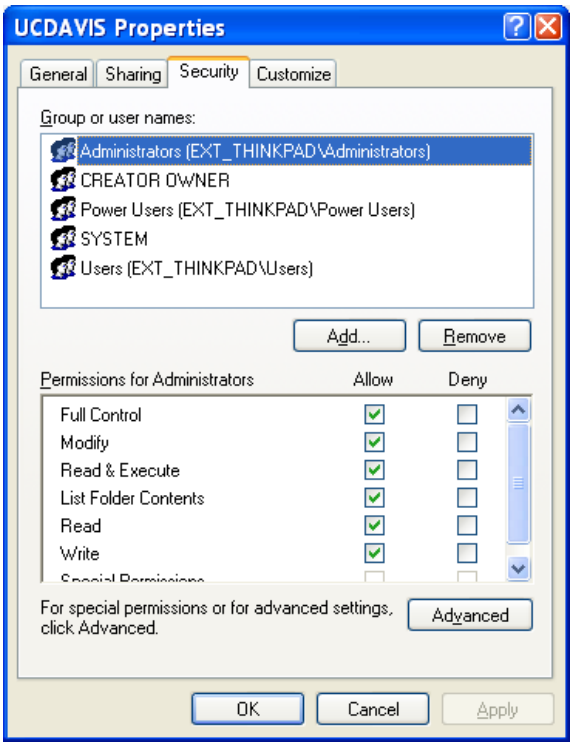
4. Click the Start menu bar. The first dialog box of Aquarius appears. Follow the prompts on the screen.

Security Permission

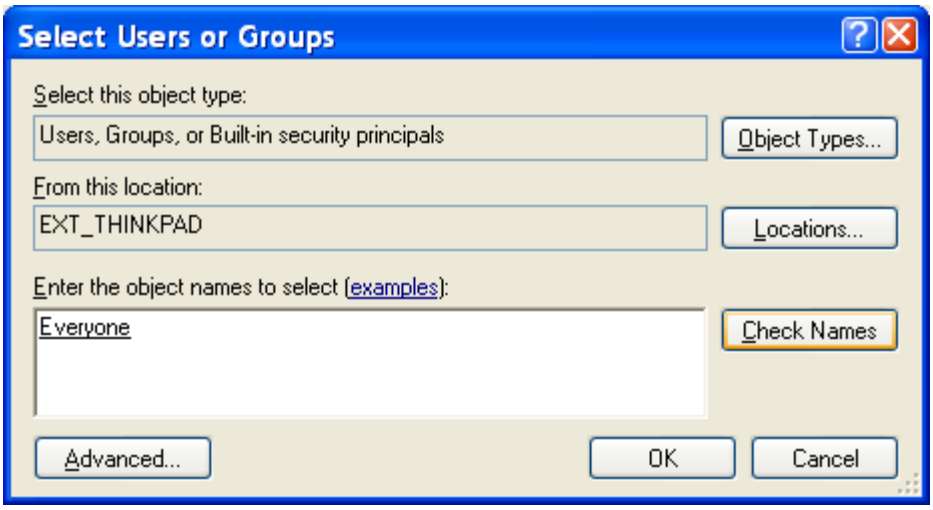
If you are going to use your computer in the non-admin mode, you must adjust the security permission of the C:\Program Files\UCDAVIS\ folder:

1. Using Windows Explore, navigate to C:\Program Files\UCDAVIS\

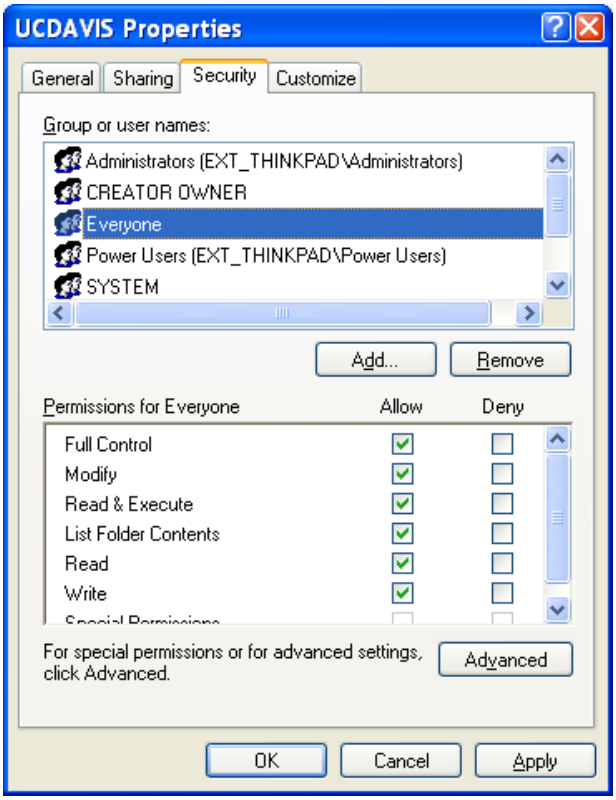
2. Right click on the folder. A menu appears. Select the Properties option. The properties dialog box appears:



3. Select the Security tab and click the Add button. Another dialog box appears:



4. In the **Enter the object to select** field, enter **Everyone** and click the **Check Names** button. The **Everyone** will be recognized and will be underlined. Click the OK button. You will back to the previous dialog box:



5. Highlight the Everyone entry and click the checkbox for Allow Full control. Click the OK button

Installing on a Drive other than C: drive

If you have installed the Aquarius program on a drive other than the C: drive, you must edit the configuration file, Aquarius.cfg, by the Notepad editor and replace all instances of C:\ with your selected drive. The Aquarius.cfg is located in the Aquarius folder at:

C:\Program Files\UCDAVIS\Aquarius2008\Aquarius.cfg

Appendix B.

Preparation of Fecal Coliform Databases for use in Aquarius 2.0

How to Prepare Databases Prior to Importing into Aquarius

Humboldt Bay is one of the five shellfish growing area in California. We will use fecal coliform and rainfall databases from Humboldt Bay as examples of how to prepare databases for use in Aquarius 2.0. Most of these databases are usually maintained in Excel spreadsheets. To prepare the databases for application in Aquarius 2.0, the Excel spreadsheet format must be converted to Comma Separated Format. Aquarius 2.0 has the flexibility that allows these databases to be exported and imported between Excel spreadsheets and Aquarius' comma separated format.

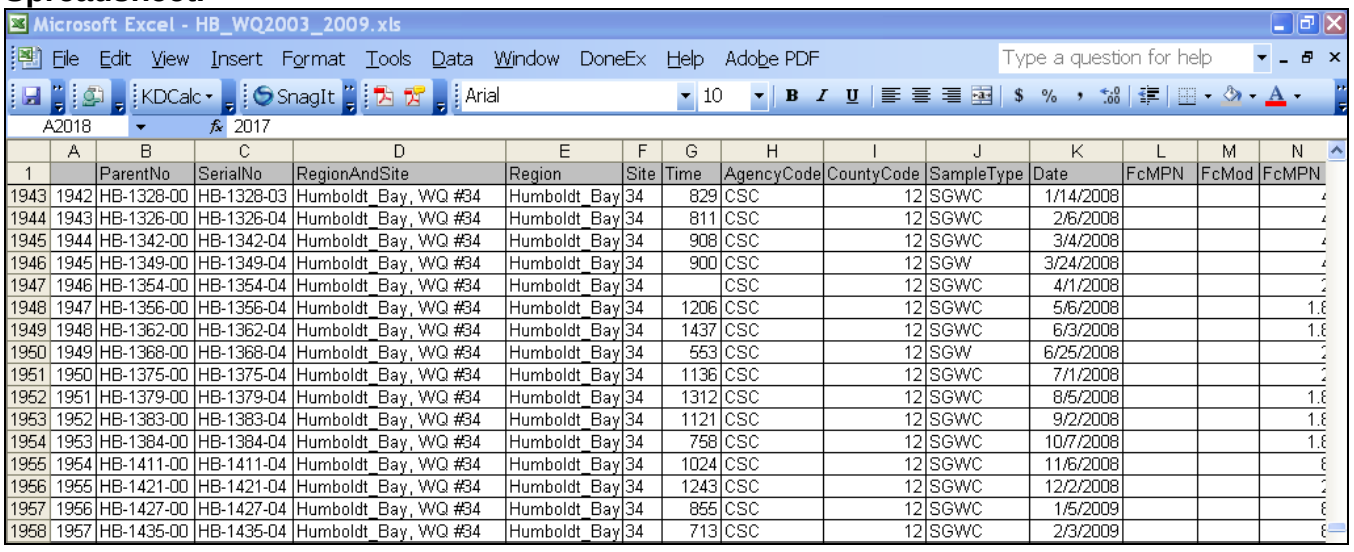
How to Prepare Fecal Coliform Data

The fecal coliform and rainfall data sets must be prepared before importing into Aquarius. To prepare the fecal coliform data for loading into Aquarius program, follow these steps:

Step 1 – Excel Spreadsheet

The Fecal coliform data is usually kept in an Excel spreadsheet, for example HB_WQ2005_2009.xls.

Figure A1 Original Fecal Coliform data for Humboldt Bay in Excel Spreadsheet.



Microsoft Excel - HB_WQ2003_2009.xls

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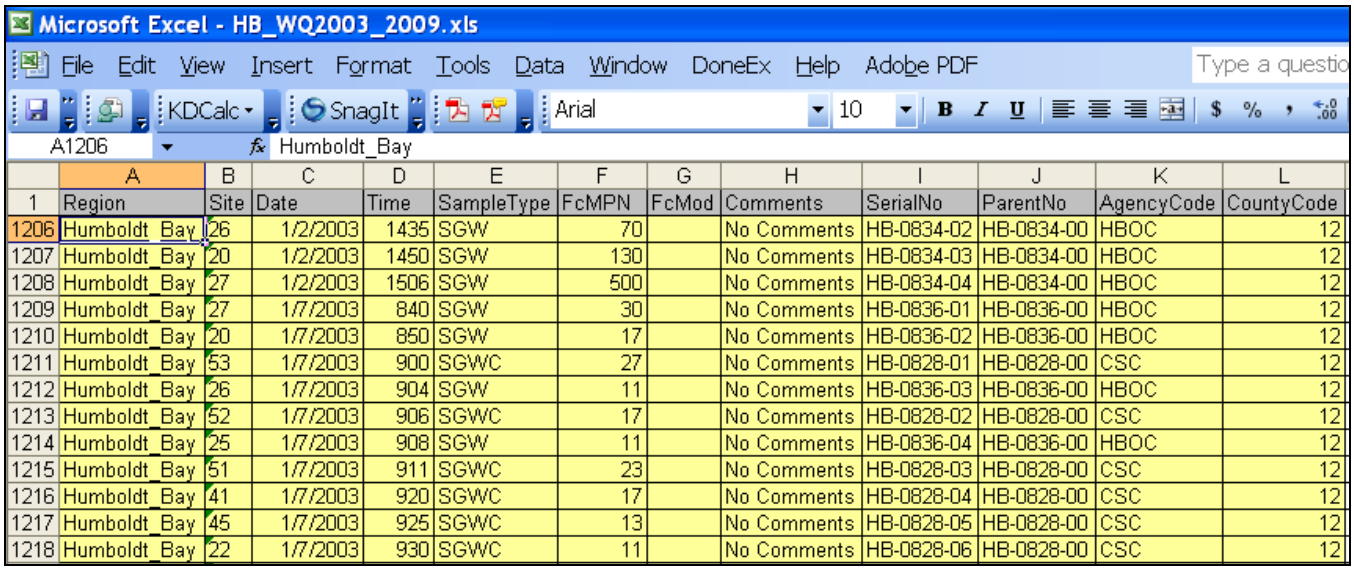
A2018 2017

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1		ParentNo	SerialNo	RegionAndSite	Region	Site	Time	AgencyCode	CountyCode	SampleType	Date	FcMPN	FcMod	FcMPN
1943	1942	HB-1328-00	HB-1328-03	Humboldt_Bay, WQ #34	Humboldt_Bay	34	829	CSC	12	SGWC	1/14/2008			4
1944	1943	HB-1326-00	HB-1326-04	Humboldt_Bay, WQ #34	Humboldt_Bay	34	811	CSC	12	SGWC	2/6/2008			4
1945	1944	HB-1342-00	HB-1342-04	Humboldt_Bay, WQ #34	Humboldt_Bay	34	908	CSC	12	SGWC	3/4/2008			4
1946	1945	HB-1349-00	HB-1349-04	Humboldt_Bay, WQ #34	Humboldt_Bay	34	900	CSC	12	SGW	3/24/2008			4
1947	1946	HB-1354-00	HB-1354-04	Humboldt_Bay, WQ #34	Humboldt_Bay	34		CSC	12	SGWC	4/1/2008			2
1948	1947	HB-1356-00	HB-1356-04	Humboldt_Bay, WQ #34	Humboldt_Bay	34	1206	CSC	12	SGWC	5/6/2008			1.8
1949	1948	HB-1362-00	HB-1362-04	Humboldt_Bay, WQ #34	Humboldt_Bay	34	1437	CSC	12	SGWC	6/3/2008			1.8
1950	1949	HB-1368-00	HB-1368-04	Humboldt_Bay, WQ #34	Humboldt_Bay	34	553	CSC	12	SGW	6/25/2008			2
1951	1950	HB-1375-00	HB-1375-04	Humboldt_Bay, WQ #34	Humboldt_Bay	34	1136	CSC	12	SGWC	7/1/2008			2
1952	1951	HB-1379-00	HB-1379-04	Humboldt_Bay, WQ #34	Humboldt_Bay	34	1312	CSC	12	SGWC	8/5/2008			1.8
1953	1952	HB-1383-00	HB-1383-04	Humboldt_Bay, WQ #34	Humboldt_Bay	34	1121	CSC	12	SGWC	9/2/2008			1.8
1954	1953	HB-1384-00	HB-1384-04	Humboldt_Bay, WQ #34	Humboldt_Bay	34	758	CSC	12	SGWC	10/7/2008			1.8
1955	1954	HB-1411-00	HB-1411-04	Humboldt_Bay, WQ #34	Humboldt_Bay	34	1024	CSC	12	SGWC	11/6/2008			8
1956	1955	HB-1421-00	HB-1421-04	Humboldt_Bay, WQ #34	Humboldt_Bay	34	1243	CSC	12	SGWC	12/2/2008			2
1957	1956	HB-1427-00	HB-1427-04	Humboldt_Bay, WQ #34	Humboldt_Bay	34	855	CSC	12	SGWC	1/5/2009			8
1958	1957	HB-1435-00	HB-1435-04	Humboldt_Bay, WQ #34	Humboldt_Bay	34	713	CSC	12	SGWC	2/3/2009			8

Create another spreadsheet as Extracted and extract the following 12 columns in the order specified into this new tab (Figure A2).

Figure A1. Extracted Fecal Coliform data for Humboldt Bay sorted on date and time.



Microsoft Excel - HB_WQ2003_2009.xls

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A1206 Humboldt_Bay

	A	B	C	D	E	F	G	H	I	J	K	L
1	Region	Site	Date	Time	SampleType	FcMPN	FcMod	Comments	SerialNo	ParentNo	AgencyCode	CountyCode
1206	Humboldt_Bay	26	1/2/2003	1435	SGW	70		No Comments	HB-0834-02	HB-0834-00	HBOC	12
1207	Humboldt_Bay	20	1/2/2003	1450	SGW	130		No Comments	HB-0834-03	HB-0834-00	HBOC	12
1208	Humboldt_Bay	27	1/2/2003	1506	SGW	500		No Comments	HB-0834-04	HB-0834-00	HBOC	12
1209	Humboldt_Bay	27	1/7/2003	840	SGW	30		No Comments	HB-0836-01	HB-0836-00	HBOC	12
1210	Humboldt_Bay	20	1/7/2003	850	SGW	17		No Comments	HB-0836-02	HB-0836-00	HBOC	12
1211	Humboldt_Bay	53	1/7/2003	900	SGWC	27		No Comments	HB-0828-01	HB-0828-00	CSC	12
1212	Humboldt_Bay	26	1/7/2003	904	SGW	11		No Comments	HB-0836-03	HB-0836-00	HBOC	12
1213	Humboldt_Bay	52	1/7/2003	906	SGWC	17		No Comments	HB-0828-02	HB-0828-00	CSC	12
1214	Humboldt_Bay	25	1/7/2003	908	SGW	11		No Comments	HB-0836-04	HB-0836-00	HBOC	12
1215	Humboldt_Bay	51	1/7/2003	911	SGWC	23		No Comments	HB-0828-03	HB-0828-00	CSC	12
1216	Humboldt_Bay	41	1/7/2003	920	SGWC	17		No Comments	HB-0828-04	HB-0828-00	CSC	12
1217	Humboldt_Bay	45	1/7/2003	925	SGWC	13		No Comments	HB-0828-05	HB-0828-00	CSC	12
1218	Humboldt_Bay	22	1/7/2003	930	SGWC	11		No Comments	HB-0828-06	HB-0828-00	CSC	12

1. **Region:** The first column shows the region. The region in the Fecal Coliform data set must match the region in the rainfall data set. No spaces are allowed. Replace space with an underscore, for example, change **Humboldt Bay** to **Humboldt_Bay**. Do not enclose region name in double or single quotes. The maximum width of this column is 30 characters. Aquarius uses this column to build a drop down list for selecting region.

2. **Site:** The second column shows the sampling site. It can be as a number or text. Aquarius uses this column to build a drop down list for site.

3. **Date:** The third column shows the date in the format of MM/DD/YYYY

4. **Time:** The fourth column shows the time in the military format hh:mm, or in the numeric format. In the numeric format, the number 732 denotes 7:32am and the number 1411 denotes 2:11pm. Aquarius can also handle military time format (hh:mm), but not the am/pm format.

5. **SampleType:** The fifth column shows the sample type. It is text. Aquarius uses this column to build a drop down list for selecting sample type.

6. **FCMPN:** The sixth column shows the Fecal Coliform in MPN per 100 ml.

7. **FCMOD**: The seventh column shows the modifier for the fecal coliform value. This column either is blank, or contains the less than sign (<), or the greater than sign (>).

8. **Comments:** The 8th column shows the Comments.

9. **SerialNo:** The 9th column shows the serial number. This is used by state agencies for tracking purposes.

10. **ParentNo:** The 10th column shows Parent No.

11. **AgencyCode:** The 11th column shows the Agency code. This is used by state agencies for tracking purposes.

12. **CountyCode:** The last column shows the County Code.

It is important to arrange the 12 columns in the exact order described above. The first seven columns are used by Aquarius, and the last five columns (columns 8 through 12) are for State Agency tracking purposes and not used in the Aquarius calculations.

Sort the data on Date (Ascending) and Time (Ascending). Ascending order is from oldest to newest.

Step 2 – Export to CVS format

From the file menu in the Excel program, select the Save As option and save the Excel file as a comma separated file with the extension *.CSV. Sometimes the Windows Explorer hides extensions for known file types and therefore you can not see the CSV extension. You must configure your windows Explorer to show extensions for known file types.

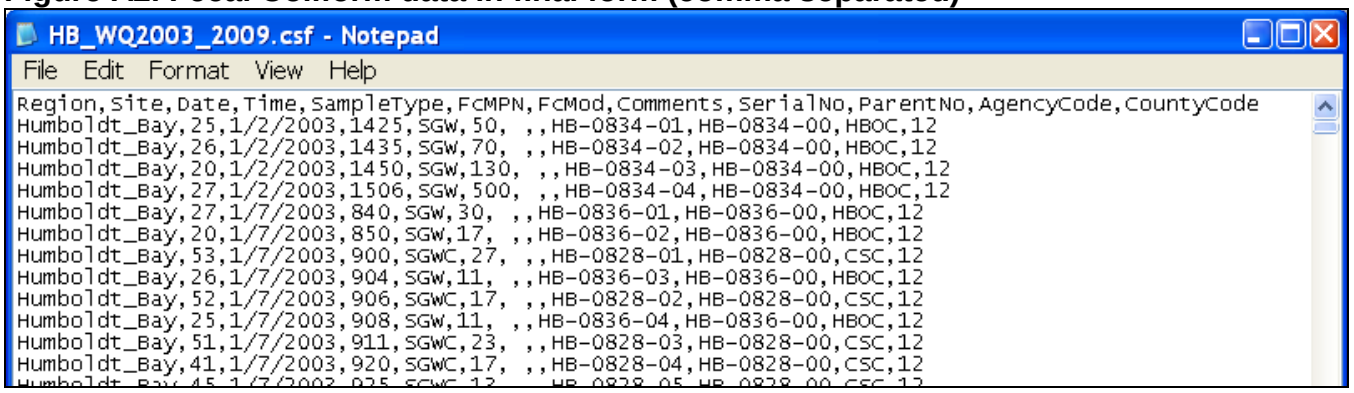
Step 3- Rename to CSF

Rename the file extension from CSV to CSF (representing Comma Separated Fecal) and copy it onto the Fecal Coliform folder of Aquarius at:

C:\Program Files\UCDAVIS\Aquarius2008\Data_FC\

The exported file looks like the following Figure A3 in the Notepad program:

Figure A2. Fecal Coliform data in final form (comma separated)



In this format, the fecal coliform database can be imported into the Aquarius program

Appendix C

How to Prepare Rainfall Databases for use in Aquarius 2.0

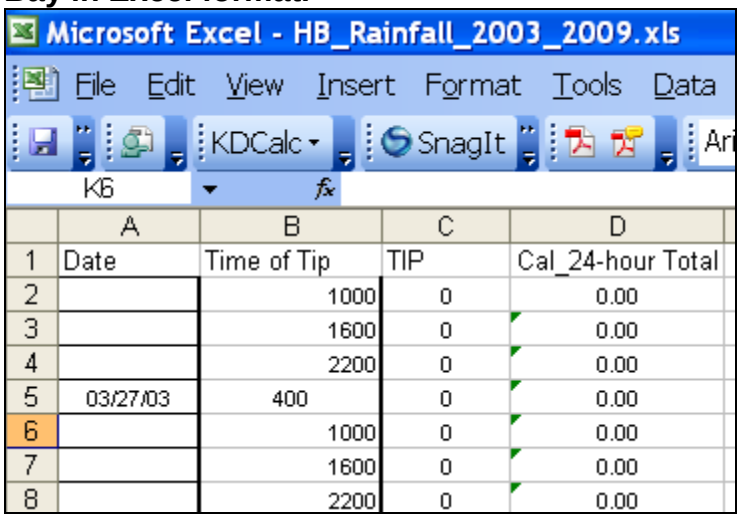
Rainfall databases are often in Excel spreadsheet format. Follow these steps to prepare rainfall data for loading into the Aquarius program.

Step 1 – Excel Spreadsheet

If the data is in hourly intervals, or 6-hourly intervals, you can easily extract the required columns; otherwise, you can use the Rainfall Processing Program (RPP) to convert the data file into Aquarius format. The RPP is specifically designed for this purpose. The RPP program can be obtained through the website http://animalscience.ucdavis.edu/extension/software/Rpp/index.htm

In this example from Humboldt Bay, the data is in 6-hourly intervals reported at 400 (4:00am), 1000 (10:00am), 1600 (4:00pm), 2200(10:00pm) for standard time and reported at 500 (5:00am), 1100 (11:00am), 1700 (5:00pm), 2300(11:00pm) for Day Time Saving (DTS):

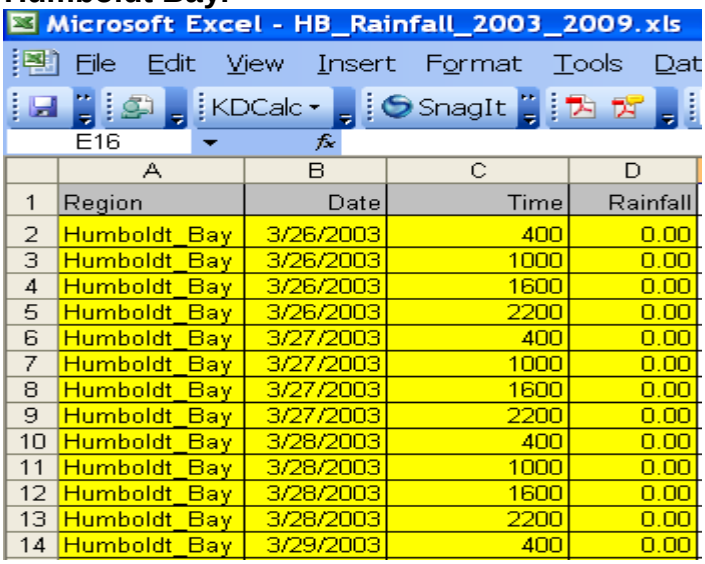
Figure B1. Original rainfall data for Humboldt Bay in Excel format.



	A	B	C	D
1	Date	Time of Tip	TIP	Cal_24-hour Total
2		1000	0	0.00
3		1600	0	0.00
4		2200	0	0.00
5	03/27/03	400	0	0.00
6		1000	0	0.00
7		1600	0	0.00
8		2200	0	0.00

Create another spreadsheet as Extracted, add a new column for region, and extract the date, time, and rainfall columns in the order specified into this new tab (Figure B2).

Figure B2. Extracted rainfall data from Humboldt Bay.



	A	B	C	D
1	Region	Date	Time	Rainfall
2	Humboldt_Bay	3/26/2003	400	0.00
3	Humboldt_Bay	3/26/2003	1000	0.00
4	Humboldt_Bay	3/26/2003	1600	0.00
5	Humboldt_Bay	3/26/2003	2200	0.00
6	Humboldt_Bay	3/27/2003	400	0.00
7	Humboldt_Bay	3/27/2003	1000	0.00
8	Humboldt_Bay	3/27/2003	1600	0.00
9	Humboldt_Bay	3/27/2003	2200	0.00
10	Humboldt_Bay	3/28/2003	400	0.00
11	Humboldt_Bay	3/28/2003	1000	0.00
12	Humboldt_Bay	3/28/2003	1600	0.00
13	Humboldt_Bay	3/28/2003	2200	0.00
14	Humboldt_Bay	3/29/2003	400	0.00

1. **Region:** The original data set does not have the Region column, so insert a new column for Region and place it as the first column. The Region column must match the Region column in the fecal coliform data set. No blank spaces are allowed in the Region name. Use underscore instead of a space or a hyphen. Do not use single or double quotes for this column.

2. **Date:** The second column shows the date in MM/DD/YYYY format.

3. **Time:** The third column shows time in the numeric format. The number 400 denotes (4:00am) and number 2200 denotes (10:00pm). To convert all Day Time Saving entries to Standard Time, use find and replace to replace 500 by 400, 1100 by 1000, 1700 by 1600, and 2300 by 2200. Aquarius can also handle military time format (hh:mm), but not the am/pm format.

4. **Rainfall:** The fourth column shows the rainfall data reported in 6-hour intervals. Aquarius prefers the hourly rainfall data, but it can handle the 6-ourl interval as well. But, if the data is in cumulative tip format with irregular intervals or if he data is in sub-hourly format with irregular intervals, then use the RPP program to convert the rainfall data into Aquarius format.

Sort the data on Date (Ascending) and Time (Ascending). Ascending order is from oldest to newest.

Step 2 – Export to CVS format

In the file menu of the Excel program, select the Save As option and save your Excel file as a comma separated file with the extension *.CSV. Sometimes the Windows Explorer hides extensions for known file types and therefore you can not see the CSV extension. You must configure your windows Explorer to show extensions for known file types.

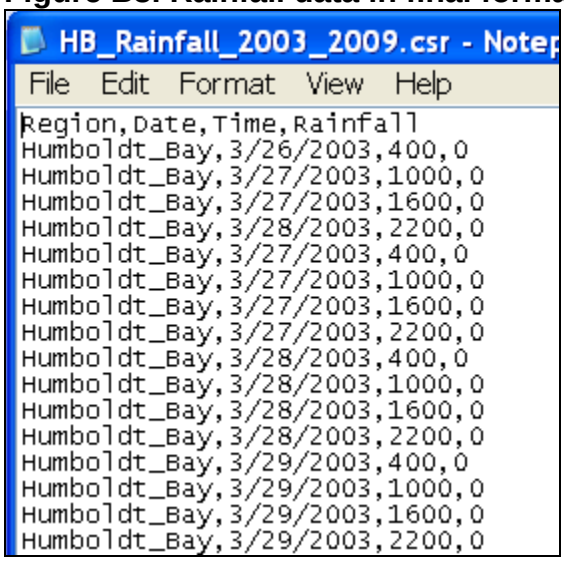
Step 3- Rename to CSR

Rename the file extension from CSV to CSR and copy it onto the rainfall folder of Aquarius at:

C:\Program Files\UCDAVIS\Aquarius2008\Data_Rainfall\

The exported file looks like the following figure in the Notepad program (Figure B3).

Figure B3. Rainfall data in final format (comma separated)



Appendix D

The Rainfall Processing Program (RPP) is a program used to process and prepare rainfall data files before the rainfall data is used in other programs and software. It consists of four modules: Dup, Rev, Sum, and Tip.

1. **The Dup module** removes the duplicate entries from a rainfall data file.

2. **The Rev module** reverses the sorting order of a rainfall data file from ascending (A to Z) to descending (Z to A), or vise versa.

3. **The Sum module** converts an hourly rainfall data file into the cumulative tip format.

4. **The Tip module** converts a cumulative tip file into the hourly format, and calculates 24 to 240 hours running cumulative rainfall values.

   The RPP program is written in C++ programming language and runs under Windows operating system. The program also supports the American and European date formats (MM/DD/YYYY and DD/MM/YYYY), and both the English and Metric systems (Inch and Millimeter)

Installation

The setup program installs the Rainfall Processing Programs in the C:\Program Files\UCDAVIS\RPP2009\ folder. After installation, the configuration files for the four modules will be located in the following subfolders:

* The configuration file for the Dup module will be in the DUP subfolder.
* The configuration file for Rev module will be in the REV subfolder.
* The configuration file for Sum module will be in the SUM sub folder.
* The configuration file for Tip module will be in the TIP subfolder.

How to run the program

Click on the “Start” button and select “All Programs”, “UCDAVIS”, “RPP2009”, and click on “Launch RPP”. The main menu of the RPP program appears.

Notepad

The RPP program uses the Notepad program to view the rainfall data files. The notepad program is usually installed in the following folder:

C:\WINDOWS\system32\notepad.exe

If your notepad program is installed in a different folder, specify its path in the rpp_cfg.cfg file. The rpp_cfg.cfg file is a text file that is located in the main folder of the RPP program. Its location is at:

C:\Program Files\UCDAVIS\RPP2009\rpp_cfg.cfg

It contains a single line in the following format:

NOTEPAD=C:\WINDOWS\system32\notepad.exe

To relocate the file, simply edit the path following the equal sign after the word NOTEPAD. For example, enter:

NOTEPAD=D:\WINDOWS\system32\notepad.exe

Then, save the file and re-start the RPP program.

NOTE: There is no space after the word NOTEPAD, or after the equal sign. Do not enclose the path in quotation marks.

Acknowledgment: The graphic in this screen is from Rainy Day painting by Marina Zavalova. Ms. Zavalova has given us permission to exhibit her work in this software. For more information about Marina Zavalova and her art, please visit her website: http://marinazavalova.com. You can also contact her at info@marinazavalova.com, or by phone at (760)207-5972.

Software Contact Information

Software Support and Distribution
Department of Animal Science
University of California
1 Shields Ave
Davis, CA 95616 USA

Email: Software@asmail.ucdavis.edu
Web: http://animalscience.ucdavis.edu/extension/software/rpp/

The Dup program deletes duplicate rows from a rainfall data file. To run the program, fill in the input fields and click the Run Dup button.

1. Input_File= Specifies the input file. There should not be any space, or special characters in the file name. The default input file is downld08.txt

2. Output_File= Specifies the output file. There should not be any space, or special characters in the file name. The default value is cleaned.txt

3. Input_Separator= Specifies the separator character between the columns in the input file. The separator character can be a COMMA, SPACE, or TAB. The default separator character is a SPACE.

4. Input_StartRow= Specifies the starting row of the actual data. The default value is 4.

5. Input_DateCol= Specifies the column for date. The default value is 1.

6. Input_TimeCol= Specifies the column for time. The default value is 2

The Rev program reverses the sorting order of a rainfall data file from [A to Z] to [Z to A] or vice versa. It keeps the column headings unchanged and reverses the body of data. To run the program, fill the input fields and click the Run Rev button.

1. Input_File= Specifies the input file. There should not be any space or special character in the file name. The default input file is downld08.txt

2. Output_File= Specifies the output file. There should not be any space or special character in the file name. The default value is reversed.txt

3. Input_StartRow= Specifies the starting row of the actual data. The default value is 4.

The Sum program converts an hourly rainfall data file into the cumulative tip format. It also deletes the duplicate entries and reverses the sorting order of a rainfall data file from [A to z] to [Z to A] or vice versa, if needed. To run the program, fill the input fields and click the Run Sum button.

1. Input_File= Specifies the input file. There should not be any space or special character in the file name. The default input file is downld08.txt

2. Output_File= Specifies the output file. There should not be any space or special character in the file name. The default value is tip.txt

3. Input_Order= Specifies the sorting order of a rainfall data file. The Valid values are ASCENDING from [A to z] and DESCENDING from to [Z to A]. The default value is ASCENDING.

4. Output_Order= Specifies the sorting order of a rainfall data file. The Valid values are ASCENDING from [A to z] and DESCENDING from to [Z to A]. The default value is ASCENDING.

5. Input_Separator= Specifiesing the separator character between the columns in the input file. It can be COMMA, SPACE, or TAB. The default separator character is SPACE.

6. Output_Separator= Specifiesing the separator character between the columns in the input file. It can be COMMA, SPACE, or TAB. The default separator character is SPACE.

7. Input_StartRow= Specifies the starting row of the actual data. The default value is 4.

8. Input_DateCol= Specifies the column for date. The default value is 1.

9. Input_TimeCol= Specifies the column for time. The default value is 2.

10. Input_RainCol= Specifies the column for rain. The default value is 11.

11. Output_Sparse= Specifies the format of output file. If the Sparse is set to YES, then the program ignores the zero rainfalls in input, and therefore does not create output files with repeated tip values. The default value is YES.

The Tip program converts a cumulative tip rainfall data file into the hourly format. It also deletes the duplicate entries and reverses the sorting order of a rainfall data file from [A to z] to [Z to A], or vice versa, if needed. To run the program, fill twenty input fields and click the Run Tip button.

Re-setting Tip counter

The tip counter can be reset to zero at any day of year.

1 Input_File= Specifies the input file. There should not be any space or special character in the file name. The default input file is tip.txt

2 Output_File= Specifies the output file. There should not be any space or special character in the file name. The default value is hourly.txt

3 Input_Order= Specifies the sorting order of a rainfall data file. The Valid values are ASCENDING from [A to z] and DESCENDING from to [Z to A].The default value is ASCENDING.

4 Output_Order= Specifies the sorting order of a rainfall data file. The Valid values are ASCENDING from [A to z] and DESCENDING from to [Z to A].The default value is ASCENDING.

5 Input_Separator= Specifying the separator character between the columns in the input file. It can be COMMA, SPACE, or TAB. The default separator character is SPACE.

6 Output_Separator= Specifying the separator character between the columns in the input file. It can be COMMA, SPACE, or TAB. The default separator character is COMMA.

7 Input_DateFormat= Specifies the format of the date column in the input file. The valid values are AMERICAN (MM/DD/YYYY) and EUROPEAN (DD/MM/YYYY) The default value is AMERICAN.

8 Output_DateFormat= Specifies the format of the date column in the output file. The valid values are AMERICAN (MM/DD/YYYY) and EUROPEAN (DD/MM/YYYY) The default value is AMERICAN.

Note: You can use the input/output date format to convert from American to European format and vise versa.

9 Input_TimeFormat= Specifies the format of the time column in the input file. The valid values are REGULAR(HH:MMAM/PM), MILITARY(HH:MM), and NUMERIC(HHMM). The default value is REGULAR.

10 Input_TimeFormat= Specifies the format of the time column in the output file. The valid values are REGULAR(HH:MMAM/PM), MILITARY(HH:MM), and NUMERIC(HHMM). The default value is MILITARY.

The following table shows the range of these formats.

REGULAR	MILITARY	NUMERIC
12:00am (Midnight)	00:00	0
1:00am	01:00	100
2:00am	02:00	200
3:00am	03:00	300
4:00am	04:00	400
5:00am	05:00	500
6:00am	06:00	600
7:00am	07:00	700
8:00am	08:00	800

9:00am	09:00	900
10:00am	10:00	1000
11:00am	11:00	1100
12:00pm (Noon)	12:00	1200
1:00pm	13:00	1300
2:00pm	14:00	1400
3:00pm	15:00	1500
4:00pm	16:00	1600
5:00pm	17:00	1700
6:00pm	18:00	1800
7:00pm	19:00	1900
8:00pm	20:00	2000
9:00pm	21:00	2100
10:00pm	22:00	2200
11:00pm	23:00	2300

Note that in the Regular Time format, there is no space between the value of time and the keyword am or pm. If in your data, there is a space between these two, then you must remove the space manually, before using this program. This rule only applies to situations when the input separators SPACE. If the input separator is TAB or COMMA, then this rule does not apply and you have the option of either using or not using a space between the value of time and the keyword am or pm.

11. Input_RainUnit= Specifies the unit of measurement for input rainfall data. The valid values are INCH and MM (millimeter). The default value is INCH.

12. Output_RainUnit= Specifies the unit of measurement for output rainfall data. The valid values are INCH and MM (millimeter). The default value is INCH.

Note: You can use the input/output rain unit to convert from Metric system to English system and vise versa.

13. Input_StartRow= Specifies the starting row of the actual data. The default value is 2.

14. Input_DateCol= Specifies the column for date. The default value is 1.

15. Input_TimeCol= Specifies the column for time. The default value is 2

16. Input_RainCol= Specifies the column for rain. The default value is 3

17. Output_Sparse= Specifies the format of output file. If the Sparse is set to YES, then the program ignores fill the gap between two tips with missing dates and times. The default value is NO.

18. Output_Cumms= Specifies to include or exclude cumulative rainfalls in the output. Cumulative rain falls are:

Running Cumulative	6-hour rainfall
Running Cumulative	24-hour rainfall (1-day)
Running Cumulative	48-hour rainfall (2-day)
Running Cumulative	72-hour rainfall (3-day)
Running Cumulative	96-hour rainfall (4-day)
Running Cumulative	120-hour rainfall (5-day)
Running Cumulative	144-hour rainfall (6-day)
Running Cumulative	168-hour rainfall (7-day)
Running Cumulative	192-hour rainfall (8-day)
Running Cumulative	216-hour rainfall (9-day)
Running Cumulative	240-hour rainfall (10-day)

The default value is NO.

19. Output_RegionCol= Specifies to include or exclude this column from the output file. The valid values are YES to include this column and NO to exclude this column. The default value is YES.

20. Output_Region= Specifies a region for the rainfall date. This name will be copied onto the first column of the output file. There should not be any space or special character in the name. In this example the region is TOMALES_BAY.

Note: If the RegionCol is set to NO, then the Region will not be add to the output file, even if a value is specified for the Region field.

The Rev program reverses the sorting order of a rainfall data file from [A to z] to [Z to A] or vice versa. It keeps the column headings unchanged and reverses the body of data. To run the program, fill the input fields and click the Run Rev button.

1. Input_File= Specifies the input file. There should not be any space or special character in the file name. The default input file is downld08.txt

2. Output_File= Specifies the output file. There should not be any space or special character in the file name. The default value is reversed.txt

3. Input_StartRow= Specifies the starting row of the actual data. The default value is 4.

APPENDIX E

Sample Size Statistics and Relationships to Analytical Procedures

Required Sample Size for the Descriptive Statistics in Sections 2 -7 of the analytical report

Aquarius calculates the required sample size twice: one time using the 95% Confidence Interval Upper Limit of the Geometric Mean, and the other time using the 95% Confidence Interval Upper Limit of the Est. 90th Percentile. The final result is the maximum of these two values.

Required Sample Size using the 95% Confidence Interval Upper Limit of the Geometric Mean

If N denotes the Sample Size, and σ denotes the sample estimate of log base 10 of the Standard Deviation, and $\bar{x}$ denotes the sample estimate of log base 10 of the Arithmetic Mean, and ***lm*** denotes the NSSP limit for Geometric Mean the for a 5-tube test or any other test, and 1.96 denotes the coefficient for 95% Confidence Interval, then:

$$Anti\log\left(\bar{x}+1.96\frac{\sigma}{\sqrt{N}}\right)<lm$$

We can use an iterative approach setting the value of N to 1, 2, 3,…,100 until the value of left side of equation become less than the right side of equation.

Required Sample Size using the 95% Confidence Interval Upper Limit of the Estimated 90th Percentile

If N denotes the Sample Size, and σ denotes the sample estimate of log base 10 of the Standard Deviation, and $\bar{x}$ denotes the sample estimate of log base 10 of the Arithmetic Mean, and ***ls*** denotes the NSSP limit for the Est. 90th Percentile for a 5-tube test or any other test, and 1.96 denotes the coefficient for 95% Confidence Interval, and CHIINV is the Excel function, then:

$$Anti\log\left[\bar{x}+1.28\sigma\left(\sqrt{\frac{(N-1)}{chiinv(0.975,N-1)}}\right)\right]<ls$$

We can use an iterative approach setting the value of N to 1, 2, 3,…,100 until the value of left side of equation become less than the right side of the equation.

Required Sample Size for T-Test Sections 8 - 11

The sample size calculation is based on the method developed in the St George's University of London. For more information please visit: http://www.sgul.ac.uk

The formula for the sample size for comparison of 2 means (2-sided) is as follows:

If ***A*** denotes the Alpha Factor, and ***B*** denotes the Beta Factor, and S_{pooled} denotes the pooled standard deviation, and ***diff*** denotes the absolute difference between two means, then the Required Sample Size for each group, ***Ng***, is:

$$N_g = 2(A+B)^2\left(\frac{S_{pooled}}{diff}\right)^2$$

Therefore the total sample size is double this value.

Alpha Factor, A, depends on desired alpha level (see table)

Beta Factor, B, depends on desired beta leve1 (see table)

If $\overline{x_1}$ denotes the mean of the first group, and $\overline{x_2}$ denotes the mean of the second group, then the absolute difference between two means, ***diff***, is:

$$diff = abs\left(\overline{x_1} - \overline{x_2}\right)$$

If n_1 denotes the observed Sample Size of the first group, and n_2 denotes the observed Sample Size of the second group, and S_1 denotes the Standard Deviation of first group, and S_2 denotes the Standard Deviation of the second group, then the pooled standard deviation, S_{pooled}, is:

$$S_{pooled} = \sqrt{\frac{(n_1-1)S_1^2 + (n_2-1)S_2^2}{n_1+n_2}}$$

Table of values for A	
Alpha	**A**
0.10	1.64
0.05	1.96
0.025	2.23
0.01	2.58

Table of values for B	
Beta	**B**
0.20	0.84
0.10	1.28
0.05	1.64

Appendix F

The GPower program is a free utility for calculating sample size and power of test.

We have been granted permission to distribute the GPower program as a free utility program along with commercial Aquarius 2.0 software by its author, Professor Dr. Axel Buchner, Institut für Experimentelle Psychologie, Heinrich-Heine-Universität, Düsseldorf, Germany. For more information about GPower program and downloading updates, please visit:

http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/

How to install GPower

To install GPower program, visit the above web site or in the Windows Explore, navigate to:

C:\Program Files\UCDAVIS\Aquarius2008\GPower_Setup folder and double click on the setup.exe.

Before running GPower

While the Aquarius program is running, write down the values of the parameters that are found at the bottom of Section 11 of this example statistical analysis as seen in the figure below:

	**** S E C T I O N 11: **********	
COMPARISON	(Old Close, New Open) VS (Old Open)	
T-Test	n1	16
T-Test	n2	24
T-Test	Lower t sub a	-2.120
T-Test	Computed t sub c	3.660
T-Test	Upper t sub b	2.120
T-Test	Alpha level	0.050
T-Test	Degree of freedom	16
T-Test	>> T-Test IS significant at Alpha = 0.050	
T-Test	>> (Old Close, New Open) IS GREATER THAN (Old Open)	
T-Test	>> n1= 16, Avg1= 0.615, Std1= 0.347, n2= 24, Avg2= 0.294, Std2= 0.067	
T-Test	>> Pooled Std= 0.218	

The statistical parameters are Alpha, beta, n1= 16, Avg1, Std1, n2, Avg2, Std2, and Pooled Std.

The alpha and beta levels are in the Region dialog box (Step 3 of 7), and the rest are in the T_Test section of statistical output (Step 7 of 7).

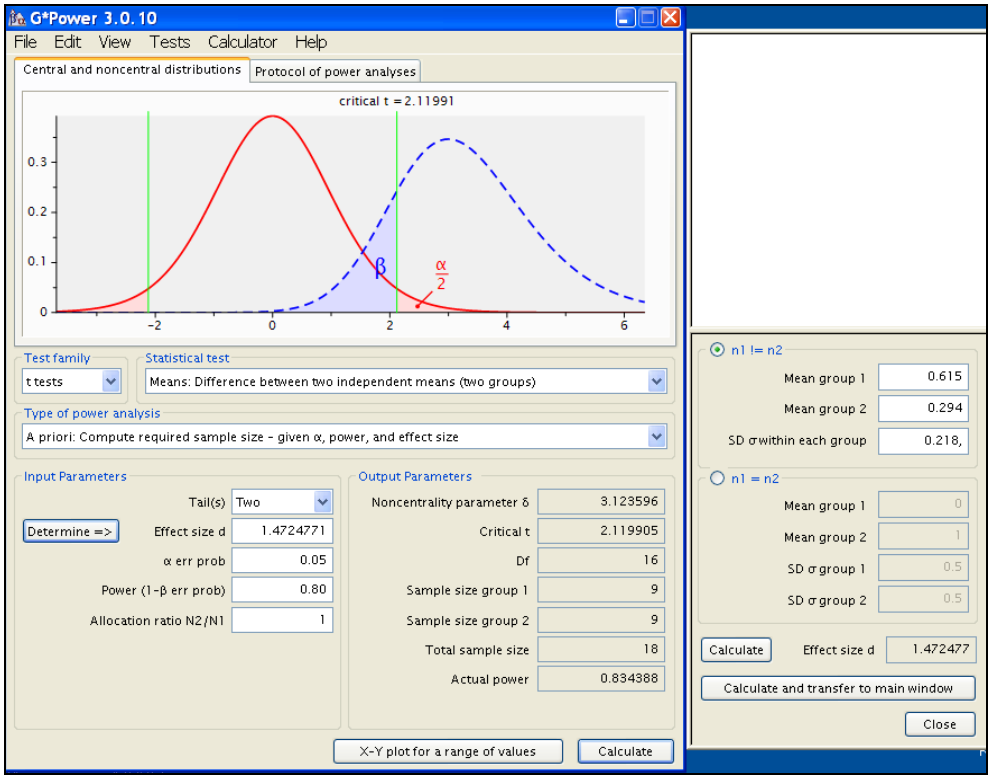
In our example:

Alpha =	0.05
Beta =	0.20
n1 =	16
Avg1 =	0.615
Std1 =	0.347
n2 =	24
Avg2 =	0.294
Std2 =	0.067
Pooled Std =	0.218

Run GPower

To run GPower program: **Press Start \ All Programs \ GPower > GPower 3.0.**

The GPower window appears:



1. For ‘TEST Family’, select ‘t tests’

2. For ‘Statistical Test’, select ‘Means: Difference between two independent means (two groups)’

3. For ‘Type of Power Analysis’, select ‘A Priori: Compute required sample size – given alpha, power, and effect size’

4. For ‘Tail(s)’, select ‘Two’

5. For ‘Effect size d’, click the ‘Determine =>’ button. A side window appears:

6. In the side window, select ‘n1 != n2’. For ‘Mean group 1’, enter the Avg1

7. From the Aquarius program. For ‘Mean group 2’, enter Avg2 from Aquarius. For ‘SD within each group’, enter the Pooled Std from Aquarius.

8. In the side window, click the ‘Calculate’ button. It calculates the Effect size d. Then click the ‘Calculate and transfer to main window’ button. It transfers the calculated Effect size d to the main window

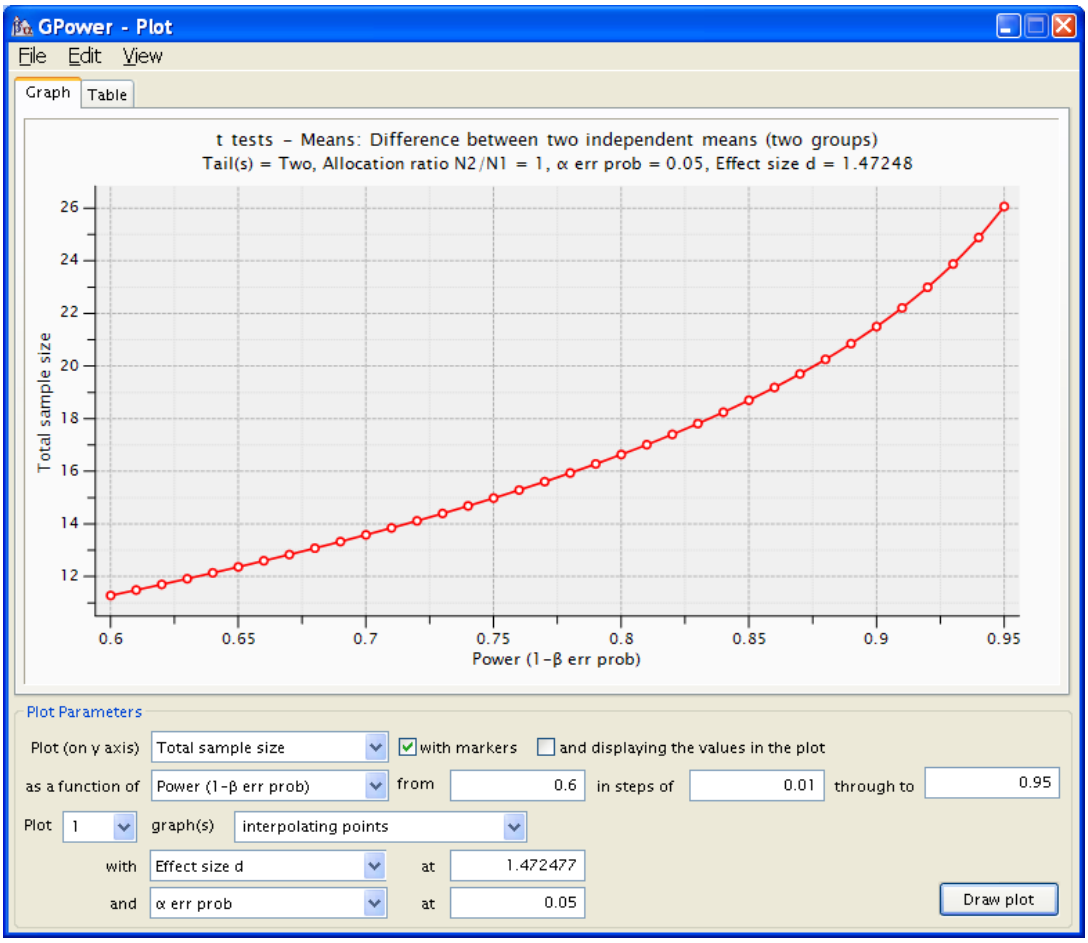
9. In the main window, for ‘Alpha err prob’, enter the alpha level from Aquarius, usually 0.05.

10. In the main window, for ‘Power (1-beta err prob)’, enter the 1- beta level from Aquarius, usually 1.00 - 0.20, or 0.80

11. In the main window, for ‘Allocation ratio N2/N1’, enter 1.

12. In the main window, click the ‘Calculate’ button. The GPower calculates the total sample size for the given power. To see a range of sample sizes for different levels of power, follow the following steps:

13. In the main window, click the ‘X-Y plot for a range of values’ button. The Plot dialog box appears. In the Plot dialog box, click the ‘Draw plot’ button. The program draws the plot of sample size over power level. See figure below.



In the Plot dialog box, changing the value of Alpha will change the graph: A smaller alpha requires more samples.

Also, changing the Effect size, changes the graph: the smaller the Effect size, requires more samples. Smaller Effect size is the result of larger standard deviation (more variability in data) or smaller the difference between two means.

Reference: Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods, 39*, 175-191.

Appendix G

Aquarius has a special tool, the **Aquarian tool**, for calculating the required sample size using the iterative method described in Appendix E.

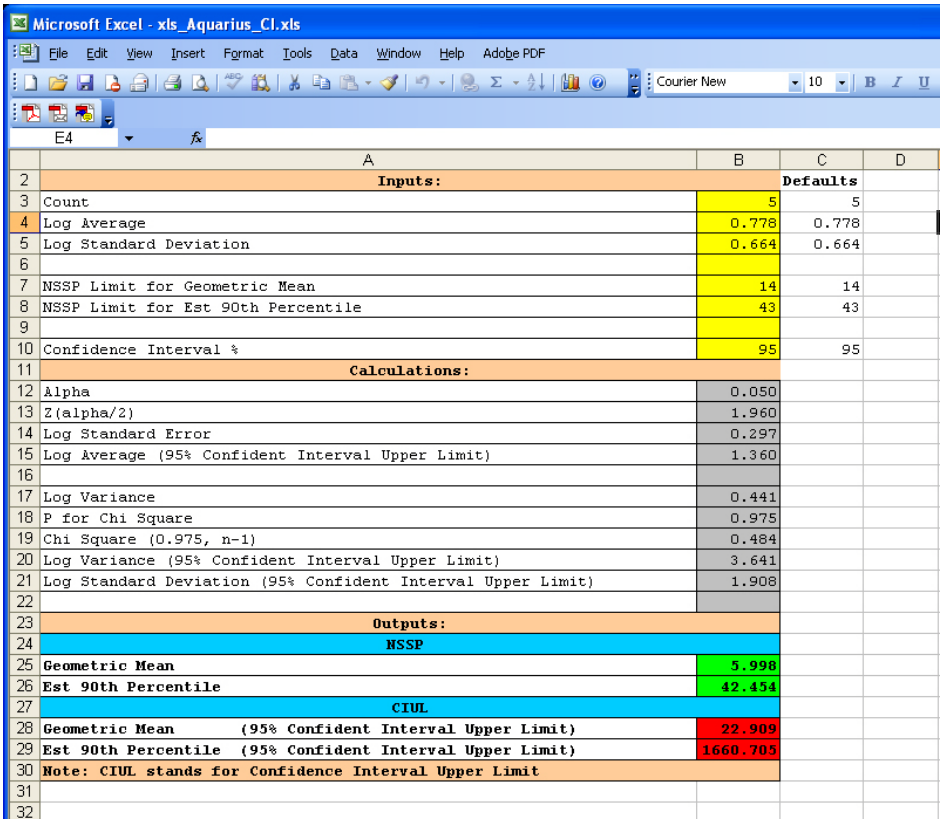
The Aquarium tool is used for testing the adequacy of the sample size using parameters described in the Descriptive Statistics Sections of the analytical report. It is based on an analytical approach developed by Peter Krottje of the California Department of Public Health and statistical formulas developed by Thomas Famula of the Department of Animal Science at the University of California Davis.

The program is designed to test the adequacy of the sample size in compliance sampling, but it is not used in rule change determination. There are times when the results meets the NSSP standard of 43, but the number of samples taken are questionable as meeting the minimum number of samples necessary to provide confidence in the results.

The Aquarian Tool is an Excel program located in the UCDAVIS folder among the Program subfolder. The file is named xls_Aquarius_CI.xls. The CI stands for confidence interval. The program determines the **Confidence Interval Upper Limits (CIUL)**

Access the UCDAVIS folder and double-click the file to open the program. The xls_Aquarius_CI.xls file appears.

Go to next page.



	A	B	C	D
2	**Inputs:**		**Defaults**	
3	Count	5	5	
4	Log Average	0.778	0.778	
5	Log Standard Deviation	0.664	0.664	
6				
7	NSSP Limit for Geometric Mean	14	14	
8	NSSP Limit for Est 90th Percentile	43	43	
9				
10	Confidence Interval %	95	95	
11	**Calculations:**			
12	Alpha	0.050		
13	Z(alpha/2)	1.960		
14	Log Standard Error	0.297		
15	Log Average (95% Confident Interval Upper Limit)	1.360		
16				
17	Log Variance	0.441		
18	P for Chi Square	0.975		
19	Chi Square (0.975, n-1)	0.484		
20	Log Variance (95% Confident Interval Upper Limit)	3.641		
21	Log Standard Deviation (95% Confident Interval Upper Limit)	1.908		
22				
23	**Outputs:**			
24	**NSSP**			
25	**Geometric Mean**	**5.998**		
26	**Est 90th Percentile**	**42.454**		
27	**CIUL**			
28	**Geometric Mean (95% Confident Interval Upper Limit)**	**22.909**		
29	**Est 90th Percentile (95% Confident Interval Upper Limit)**	**1660.705**		
30	**Note: CIUL stands for Confidence Interval Upper Limit**			
31				
32				

The program is made up of three primary windows: Input, Calculations, and Output.

The **Input Window** is accompanied by a Yellow cell section on the right containing three input values for Count, Log Average and Log Standard Deviation. Below the first three entries in the Yellow cells are the NSSP Limit for Geometric Mean, the NSSP Limit for the 90th percentile, and the Confidence Interval percentage. The default values are maintained in the column to the right of the Yellow cells.

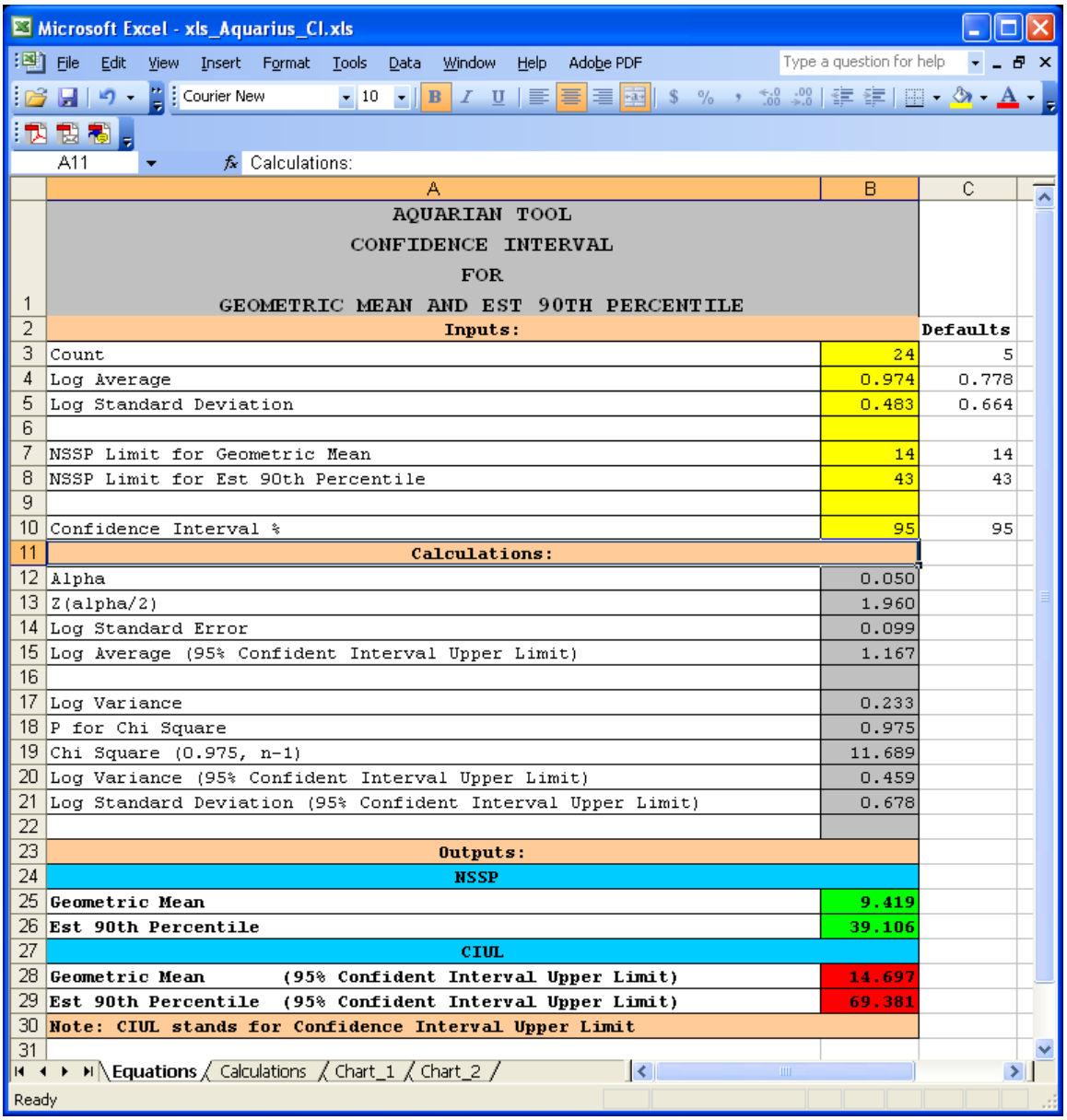
To run the program, the values for Count, Log Average and Log Standard Deviation are obtained from the Descriptive Statistics Sections of the output analysis.

The **Calculations Window** contains nine statistical parameters that are calculated based on the values that are entered into the **Input Window**. This calculation is automatic, when the values are entered into the Input Window.

The **Outputs Window** contains two displays: the NSSP Geometric Mean and the NSSP Estimated 90th percentile; and the CIUL Geometric Mean (95% CIUL) and the CIUL Estimated 90th Percentile (95% CIUL).

Example Run

Using results from Example 4 in the manual, the values for Count, Log Average, and Log Standard Deviation are 24, 0.974, and 0.483 respectively. When entered into the Aquarian Input Window, the screen appears as follows:



Microsoft Excel - xls_Aquarius_CI.xls

File Edit View Insert Format Tools Data Window Help Adobe PDF — Type a question for help

Courier New 10

A11 Calculations:

	A	B	C
1	AQUARIAN TOOL CONFIDENCE INTERVAL FOR GEOMETRIC MEAN AND EST 90TH PERCENTILE		
2	**Inputs:**		**Defaults**
3	Count	24	5
4	Log Average	0.974	0.778
5	Log Standard Deviation	0.483	0.664
6			
7	NSSP Limit for Geometric Mean	14	14
8	NSSP Limit for Est 90th Percentile	43	43
9			
10	Confidence Interval %	95	95
11	**Calculations:**		
12	Alpha	0.050	
13	Z(alpha/2)	1.960	
14	Log Standard Error	0.099	
15	Log Average (95% Confident Interval Upper Limit)	1.167	
16			
17	Log Variance	0.233	
18	P for Chi Square	0.975	
19	Chi Square (0.975, n-1)	11.689	
20	Log Variance (95% Confident Interval Upper Limit)	0.459	
21	Log Standard Deviation (95% Confident Interval Upper Limit)	0.678	
22			
23	**Outputs:**		
24	**NSSP**		
25	**Geometric Mean**	**9.419**	
26	**Est 90th Percentile**	**39.106**	
27	**CIUL**		
28	**Geometric Mean (95% Confident Interval Upper Limit)**	**14.697**	
29	**Est 90th Percentile (95% Confident Interval Upper Limit)**	**69.381**	
30	**Note: CIUL stands for Confidence Interval Upper Limit**		
31			

Equations / Calculations / Chart_1 / Chart_2 /

Ready

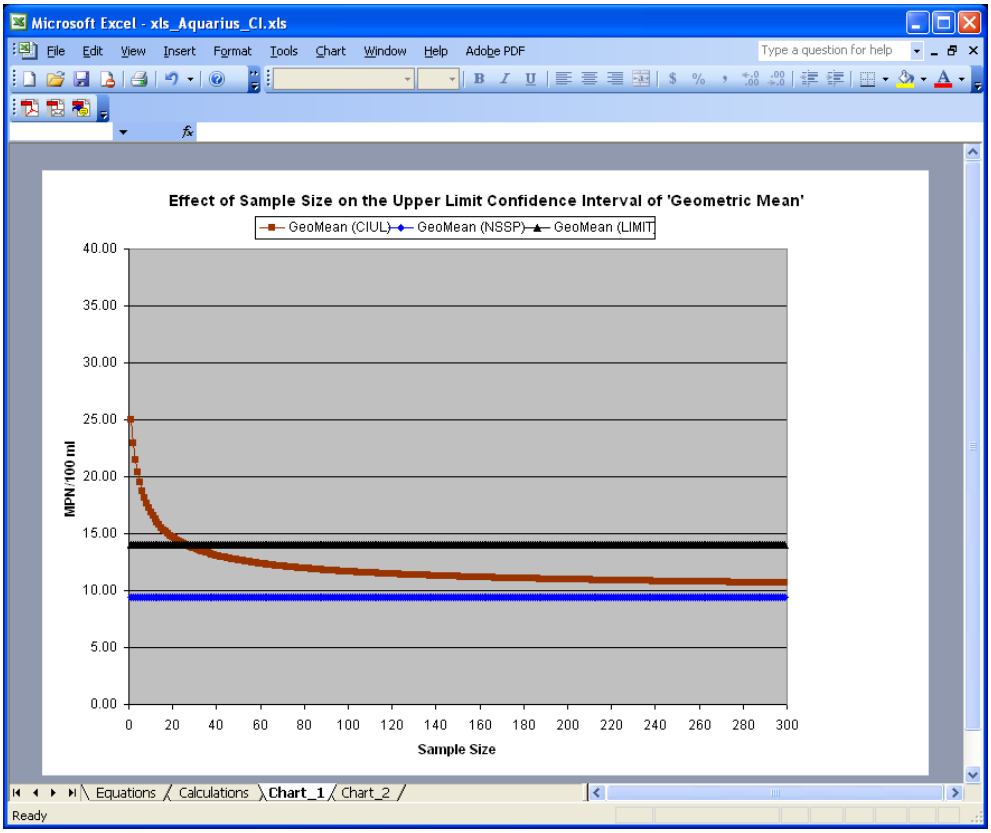
The program automatically calculates two sets of values. The first set is the Geometric Mean and the Estimated 90th percentile using the NSSP original calculations. In this example, the Geometric Mean is 9.419, which is less than the limit of 14, and the Estimated 90th percentile is 39.106, which is less than the limit of 43. Both of these values comply with the original NSSP requirements and therefore the program displays them with a green background. However, if we use the observed sample size of 24 to enhance the original NSSP calculation, we

will get two different sets of values, which are shown in the above figure under the title **CIUL**. In this example, the enhanced Geometric Mean is 14.697, which does not comply with NSSP requirements, and enhanced Estimated 90th percentile is 69.381, which also does not comply with NSSP, and therefore the program displays them with a red background.

This example clearly shows that in certain circumstances, especially when the Geometric Mean and the Estimated 90 Percentile are close to the NSSP limits, it may be exhibiting compliance, but actually the sample size may be inadequate.

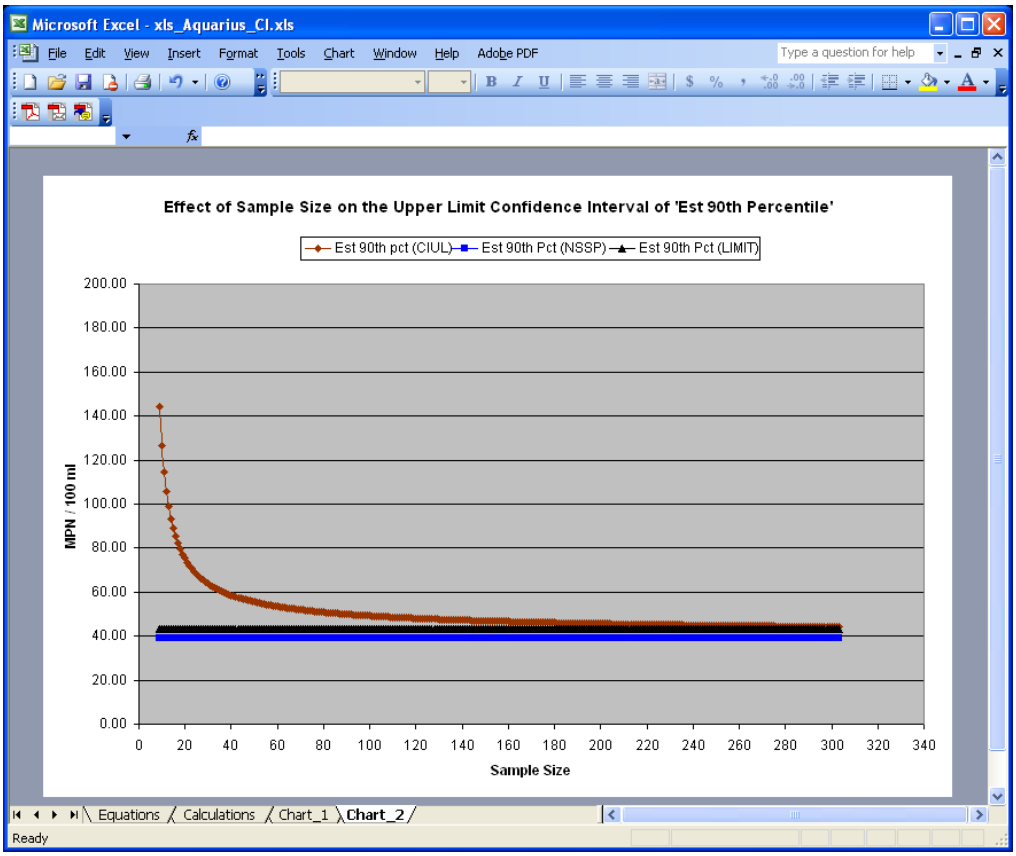
To investigate the effect of sample size in calculating the Geometric Mean, we go to Chart 1. In this chart, the x-axis shows the sample size from 0 to 300, and the y-axis shows the Geometric Mean. There are three lines in the chart. The black line displays the NSSP limit for geometric mean (14). The blue line displays the calculated Geometric Mean of this example. The red line displays the enhanced Geometric Mean.

Note that the red line crosses the black line at about sample size 30. Below 30, the enhanced Geometric Mean is above the NSSP limit and above 30 the enhanced Geometric Mean is below the NSSP black line. Our observed sample size is 24, and therefore the enhanced Geometric Mean is above the NSSP limit. To have confidence in the GM calculation, we have to have at least 30 samples.



To investigate the effect of sample size in calculating Estimated 90th Percentile, we go to Chart 2. In this chart, the x-axis shows the sample size from 0 to 340, and the y-axis shows the Estimated 90th Percentile. There are three lines in the chart. The black line displays the NSSP limit for Estimated 90th Percentile (43). The blue line displays the calculated Estimated 90th Percentile of this example. The red line displays the enhanced Estimated 90th Percentile.

Note that the red line never crosses the black line, even at a sample size of 340. The blue line clearly is below the NSSP limit, but the red line is always above the NSSP black line. This tells us that the sample sizes may not be obtainable.



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***** The Rainfall Processing Program (RPP) is valuable and useful program for the preparation of rainfall data prior to its use in any rainfall software program, including Aquarius v.2.0. It consists of four modules: Dup, Rev, Sum, and Tip.

1. The Dup module removes the duplicate entries from a rainfall data file.
2. The Rev module reverses the sorting order of a rainfall data file from ascending (A to Z) to descending (Z to A), or vise versa.
3. The Sum module converts the individual tips into the cumulative tips.
4. The Tip module converts the cumulative tips into the hourly format, and calculates 24 to 240 hours running cumulative rainfall values.

The RPP program is written in C++ programming language and runs under Windows operating system. The program also supports the American and European date formats (MM/DD/YYYY and DD/MM/YYYY), and both the English and Metric systems (Inch and Millimeter).

For information how to obtain the Rainfall Processing Program, refer to Software Support and Distribution located on the front page of this manual.

NOTES