

Project AQ3: "Manual for Regional Shrimp
Farming Environmental Monitoring and Water
Quality Training"

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H u r r i c a n e M i t c h R e c o n s t r u c t i o n P r o g r a m

Preface

This document is the English language version of the Manual for Regional Shrimp Farming Environmental Monitoring and Water Quality Training that is also available in Spanish.

As part of the participation of Auburn University in the Hurricane Mitch Reconstruction Program of the U.S. Department of Agriculture (USDA) in 2001, Auburn University conducted a training course covering key ideas in water quality management, water quality variables, and the monitoring of water quality variables. The course was designed for a period of four days, and was aimed at twenty to thirty participants. The course was given twice, once in Honduras and once in Nicaragua, and included a follow-up consultation. The course was taught primarily by Dr. Claude E. Boyd, who was assisted by several graduate students and the cooperation of agencies from the host countries. This manual presents key topics in water quality, the important variables, and the issues associated with the monitoring of the key water quality variables. This publication can be used apart from the course as a reference on water quality management and laboratory topics, but is developed primarily for skilled instructors who conduct training courses on topics related to water quality monitoring and shrimp farming.

The role of shrimp farm effluents in estuarine water quality

In order to assess the effect of shrimp farm effluents on the quality of water in a receiving water body, it is best to get baseline measurements of the water quality in the receiving water body. One also should get periodic measurements of the water quality in the receiving water body to see how water quality has changed.

Even with good measurements of all the relevant variables for the water in the receiving body, it is not usually possible to differentiate clearly between the effects of shrimp farm effluents from the effects of other effluents on the water quality of the receiving body. Sometimes the best that can be done is to assess the quality and quantity of shrimp farm effluents, and to make sure those stay within accepted guidelines. If they do stay within accepted guidelines, and the water quality of receiving bodies deteriorates, then planners might have to evaluate all possible sources of pollution, including urban, agricultural and manufacturing wastes.

Need to determine quality and volume of effluents

To assess the effect of shrimp farming effluents on receiving bodies of water, it is necessary to measure both the quality and the quantity of shrimp farm effluents at given times over a year. Quality and quantity interact, so that knowledge of neither alone is enough. A large quantity of a poor quality effluent can cause more of a problem than a small quantity of a bad quality effluent. From samples taken over the course of a year may be determined the approximate average quality of shrimp farm effluents over the year, and their total quantity.

Sometimes it is difficult to determine the quantity of shrimp farm effluents directly even by carefully planned sampling at distinct points. The quantity can be estimated by calculating the amount of water in production in ponds and the extent to which that water is turned over per year (see end of this document). Also, it might be possible to estimate the quantity of effluents indirectly by assessing the quality of effluents and their effect on the receiving

body of water, if the size of the receiving body of water is known.

The number of variables chosen

It is not possible to measure every variable that might be important for water quality issues. Measurement should be taken of variables mandated by law. Variables that are deemed important by farmers, local people or ecologists also should be measured. The most important variables are those that are most likely to cause deterioration in water quality. These most important variables appear below in Table 1, along with the reasons for measuring them and some suggested guidelines for interpreting which changes are likely to have impacts on the ecosystem. Some other variables might be included but experience has indicated that it is not crucial also to measure these other variables: nitrate-nitrogen, soluble reactive phosphorus, chemical oxygen demand, particulate organic matter, volatile solids, oil and grease, settleable solids, and turbidity. Depending on the available financial resources, it is probably better to select a few variables that are most important for the local area and to get very good measurements of those rather than to try to cover many variables less accurately. If a variable is particularly important at a specific site, of course it should be measured.

Table 1. Recommended Variables for Water Quality Monitoring

VARIABLE	REASON FOR MEASURING	GUIDELINES FOR PROTECTING AQUATIC ECOSYSTEMS
Water temperature	Has marked influence on chemical and biological activities	Less than 2 degree centigrade change
Dissolved oxygen	Essential for aerobic aquatic life	Remain at not less than 5 mg/L
pH	Influences chemical and biological processes	6/0 to 9.0

Total ammonia nitrogen	Plant nutrient and potential toxin, indicator of pollution	Should not exceed 3 mg/L in effluents
Nitrate nitrogen	Potential toxin	Should not exceed 0.005 mg/L in coastal waters
Total phosphate	Source of soluble inorganic phosphorus for plants	Concentrations of 0.001 to 0.1 mg/L in coastal waters can cause plankton blooms
Total nitrogen	Source of dissolved inorganic nitrogen for plants	Concentrations of 0.1 to 0.75 mg/L in coastal waters can cause plankton blooms. Should not exceed 10 mg/L in effluents.
Chlorophyll a	Indicator of phytoplankton abundance and degree of eutrophication	Concentrations above 1 to 10 mg./L indicative of eutrophication in coastal waters
Total suspended solids	Indicator of suspended soil particles or suspended organic matter	Should not cause a change of over 10% of seasonal mean in coastal waters
Biochemical oxygen demand	Indicator of organic pollution	Should not depress dissolved oxygen concentrations below 5 or 6 mg/L
Salinity	Can cause salinization	Should not increase salinity more than 0.5 ppt in freshwater. No limit recommended for marine or brackish waters.
Secchi disk visibility	Index of water clarity or turbidity	Should not change by more than 10% of

		seasonal mean in coastal waters
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Placement and number of water sampling sites for monitoring receiving water body quality

Water should be taken from sites that represent the average water quality in the receiving water body. If the receiving water body is of different depths and/or has qualitatively different zones, then samples should be taken from the important various areas within it. For example, if a receiving water body has both a deep center and a marshy periphery, samples should be taken from both areas. It might be necessary to set up a stratified random sampling system if the results need to be statistically significant for scientific reasons or to satisfy regulations. Samples should be taken from the stratum (depth) which best represents the biotic character of that area of the water body. A sample from deep water should probably be taken about a meter (or more) below the surface, but not necessarily several meters deep. A sample from a shallow area should be taken between the surface and the bottom. Care should be taken not to disturb the bottom sediment and thereby to accidentally include it in the water sample. Enough samples of different areas should be taken to represent the ecosystem of the receiving water body and to satisfy statistical or regulatory needs. Usually it is not necessary to take more than one sample from a distinct area within the receiving water body because multiple samples from the same area do not add much to statistical significance.

Sites should not be located in areas which are not representative or which present particular problems, such as pollution "hot spots" or where unusually fresh water enters the body (directly at the mouth of a completely unpolluted stream). Samples should not be taken if conditions clearly differ from normal for short-term reasons, for example if the water is particularly turbid due to unusual wind action. If samples are taken from unusual sites, or under unusual conditions, they should be labeled clearly as such. The role that such samples play in analysis depends on the purposes in analysis, but where they are included in analysis that inclusion should be made clear in reports.

For long-term assessments of water quality, sediment samples might need to be taken. These samples also should be taken from a full variety of representative areas in the receiving water body. The exact method for taking them depends on the time-depth of information required from the core, so that details of the methods are beyond the scope of this manual.

Placement and number of water sampling sites for monitoring effluent water quality

From where samples of effluent are taken depends on the purposes for taking the samples. If the purpose is to assess which Better Management Practices (BMPs) should be adopted, or how well they are working, then the samples need to be taken as close to the farm inlet sources and outlet points as possible. The samples need to be taken near the pumping station and near to the pond outlet mouths. It might be possible to take water from a joint outlet canal that serves several ponds or farms.

If the purpose is to assess the probable effect of effluents on the receiving water body, then the samples should be taken from areas as representative as possible. Samples should be taken (1) near the point where effluents enter the receiving water body such as the outpoint of drainage canals, drainage pipes or water gates; (2) where effluents are likely to be well mixed with waters from the receiving body; and (3) from well within the central portion of the various types of areas in the receiving water body (such as in deep central water, marshy fringes, areas of internal currents, along the sea shore, along sandy beaches, near areas of settlement, etc.). If effluents pass man-made settling basins or natural swampy areas that serve as settling basins ("green belts") then (4) samples should be taken from these areas as well. Samples should be taken from (5) near all the major shrimp producing areas that border the receiving body of water, and from (6) near all the large farms. If water is stratified at any site and the stratification makes a difference, then it might be necessary (7) to take water from several different depths. For example, it might be necessary to take water from near the surface and near the bottom to get data on differences in dissolved oxygen. For statistical or regulatory reasons, it might be necessary to approximate a stratified random sampling design or other statistically recognized design. It is usually necessary to take only one sample from a

sampling site at a time since multiple samples do not add much to accuracy or to statistical significance.

Samples should not be taken from known "hot spots" or trouble areas such as sewage outlets or factory outlets. Samples should not be taken from areas where pollution is known to collect, from areas of known eutrophication (unless that is a wide-spread condition in the receiving water body), or from areas of known long-term contamination. If samples are taken from these areas, they should be distinctively labeled as such. Samples from such areas should be used in statistical or regulatory evaluation only for a definite reason, and their use in such analyses should be made explicit in reports.

Often it is not possible to take samples completely randomly or from every important area within a receiving water body. Sometimes samples can only be taken from some public areas or from near farms whose owners have given explicit permission. Some areas might be physically inaccessible, or it might cost too much money to sample some sites regularly. In these cases, samples should be taken from as many places as practical. If possible, take samples from enough different sites so that some statistical tests can be conducted, perhaps samples from at least twelve different sites.

Methods of collecting water samples

Enough appropriate sample containers should be prepared well beforehand. The best containers are usually 0.5 liter, 1 liter or 2 liter bottles made of glass or of high-density polypropylene. Bottles should not be rinsed with anything that might contaminate the sample. Often, tap water can contaminate a sample. The final rinse should be with distilled water or de-ionized water, and the bottles should be allowed to dry thoroughly. For highly sensitive tests, the bottles should be baked at 450 degree C. before use.

All samples should be taken on the same day if possible, and as near to the same time on the day as possible. For example, take all samples in the morning of the same day. Samples should be taken at regular intervals. Once a week is good, but twice a month might be more practical. All sampling for a biweekly or monthly period should be done within the same week at the longest. For deep water, a 1-

meter column sampler can be used. (These are long tubes of various design. The specific design used depends primarily on the preference of the people taking the samples.) For shallow water, a dipping bucket can be used. Some measurements should be taken when the sample is collected, such as of temperature and dissolved oxygen.

At the time of collecting, the collecting device (a tube or dipper) should first be thoroughly rinsed in the sample water. Then the sample bottle should be thoroughly rinsed in the sample water. Only then can the sample bottle be filled and, if necessary, the sample treated.

When a sample is taken, the nature of the sample, the time and date, the place, and the person taking the sample should all be recorded on the sample bottle and in a separate notebook. A paper notebook should be kept even if this information will again be entered into a computer. Any unusual circumstances also should be noted, such as if it was raining when a surface sample was taken, or if the water had been stirred into unusual turbidity, or if a long-term rain had likely diluted the sample with fresh water.

In deep water, samples should be taken all from the same depth if possible (usually about a meter below the surface). Sometimes, at some sites, it might be useful to take the water from a particular depth that differs from the usual depth elsewhere. In that case, the sample should always be taken from the same depth at that particular site. The depth at which the sample is taken should be recorded for all sites.

When using a boat to collect samples, be careful that the boat itself is not a source of contamination. Contamination can come from the engine area (exhaust or oil), from the hull, or from material within the boat (food). If necessary, wash the boat before going out to collect samples.

Preservation of water samples

At the time of collection, samples should be placed on ice and cooled to within 0 to 4 degrees C. At that temperature, most of the variables listed in Table 1 remain stable for up to one week. However, samples should be analyzed within 24 hours of collections, or at most within 48 hours of

collection. Some variables can be stabilized by the addition of 1 ml. of concentrated H_2SO_4 per liter at the time of collection (be sure that the bottle can withstand this addition). These variables include chemical oxygen demand, ammonia and nitrate. Total or calcium hardness can be stabilized by adding 1 ml. per liter of HNO_3 . Usually it is not a good idea to add both stabilizing acids to the same sample bottle. For some variables, such as some phosphates, acid should not be added. Consult a detailed manual. The researchers should be clear as to which variables are important and follow the proper procedures for those particular variables, as indicated in relevant manuals. Different bottles may have to be prepared to isolate different groups of variables and treat them appropriately.

Laboratory storage/maintenance of water samples

The laboratory should maintain a log book (record book or note book) for samples, a book that is distinct from the one used when taking samples in the field. When samples arrive in the laboratory, they should be recorded in the laboratory log book. If a unique code number had not been given to the sample at the time of collection, one should be given when the sample is recorded in the laboratory. For work that might have legal implications, it is best to have a second person check the labeling and recording in the record book, and to indicate in writing that he/she has done the checking. If this information is recorded in a computer, a second person should check the computer entries.

The samples should be kept in a refrigerator. If possible, all the bottles from one sample site, and all the sites from one sampling period, should be kept together, although it might be necessary to separate bottles treated differently.

Some samples need to be filtered, although the major variables listed in Table 1 do not usually require filtering to process. In case filtering is required, a large enough sample should have been taken so that an unfiltered reserve backup can be kept as well. The unfiltered reserve backup and the filtered sample should be kept together if possible, but marked to clearly differentiate them. The date, time, procedure and identity of the technician should be noted on the bottle of the filtered sample and in the laboratory record book, and that information double-checked in the laboratory record book by someone else.

Water samples probably should not be kept longer than a month unless it is necessary to do so for legal reasons. Periodically, old samples should be removed from the refrigerator. Ideally, samples should be discarded once analysis is done unless technicians have reason to believe that analysis might need to be repeated. The longer a sample is kept, the more it deteriorates, and the less reliable is analysis.

Quality control: accuracy and precision

Analytical results should be both accurate and precise. Since these two concepts can be confused, it is best to review them. Suppose that the true salinity of some water is 35.0 parts per thousand (ppt). Suppose that one technique to measure the salinity of a sample leads to a mean result of 34.8 ppt when repeated over many trials; the technique could be described as fairly accurate. However, if the variation (variance) in results between repetitions in testing is large enough, the variation could cause us to mistrust the accuracy, or the variation might lead to statistically weak results despite the accuracy. Suppose another technique leads to a result of 33.1 parts per thousand very consistently when repeated, with little variance between repetitions. This second technique is not as accurate as the first technique but it is more precise. The precision is desirable but it can lead to misleadingly high statistical reliability for a-not-fully accurate answer. The high precision also can lead us to misplace confidence in the technique. Of course the ideal is both high accuracy and high precision, but it is not always possible to achieve that ideal.

The "true" value for any variable cannot be found directly without using some measuring technique, so it is not possible to achieve complete certainty. Still, methods have been developed to check accuracy and precision, and to make techniques as precisely accurate as possible. Essentially, these methods involve comparing repeated measurements in various ways (and see below). It is not possible here to describe the various ways of checking. The reader should refer to a manual. It is not necessary to carry out checks of accuracy and precision with each technique for each variable for each sample. It is probably enough to carry out checks once every month or every three months for each

technique for each variable. These checks should be regularly scheduled and the schedule should be followed.

Quality control charts should be maintained for each variable, preferably for each variable for each sampling site. In brief, a quality control chart is a two-dimensional graph in which time is on the x-axis and the results of tests are on the y-axis. These charts allow rapid recognition of deviations and of trends, and thus allow a "first-line" of rapid recognition of possible errors. It is useful to make two charts for each variable, or to plot two colors on the same chart for each variable. One color marks the mean on the y-axis while the other marks the variance on the y-axis. Values for the mean help point out problems with accuracy while values for the variance help point out problems with precision. The exact techniques for using statistical analysis on the charts can be found in manuals. Since analysis over time is likely to be a major goal of the laboratory anyway, these charts will help with other laboratory work and should not be viewed as a hindrance.

One common method of checking accuracy and precision consists of the following: (1) Make repeated measurements on a sample; then (2) make standardized changes in concentration of the sample with clean, de-ionized water (or other suitable solvent); finally, (3) make repeated measurements on the new samples that result from the various standard changes in concentration. The changes in concentration come from diluting the original sample might be to 0.50, 0.10, and 0.01 of its original concentration. The concentration can also be increased by evaporation if the evaporation can be accurately controlled (for example to 2.0 or 10.0). If the measurement technique is sufficiently accurate and precise, the results of tests on the new concentrations, and the amount of variance, should be predictable. Deviations indicate problems in accuracy or precision or both. Details of this method, its variants, and the statistical verifications, can be found in reference manuals.

Quality control, other issues

The bases for good quality control are (1) well-recognized standardized procedures, a (2) record-keeping system, and (3) faithful adherence to both. This manual cannot describe

in full either standard procedures or record-keeping, for which the reader is referred to standard texts.

The standard procedures suggested here have been verified by experience at many laboratories and for many purposes. So as to make comparison between sites and laboratories easier, it is best if these procedures are followed as much as possible. Even if other procedures are followed, it is probably best not to use test kits since such kits are not usually as reliable as well-known procedures carried out by reliable people in a well-run laboratory.

As mentioned above, initial registration of a sample should have occurred when it was taken. The delivery of the sample should additionally be recorded when it arrives at the laboratory. Someone else should check to make sure that the sample was correctly recorded in the field and when it was received. If the initial information and the delivery note are recorded in a computer, someone should check the computer entry.

In a similar manner, one person should record the results from analyses as they come in, then his/her recordings should be verified by another person, who notes his/her own verification in writing. Even when the results are recorded in a computer, the computer entries should be checked by someone other than the person who entered them. It might not be necessary to do full double verification if the results will not be used to satisfy legal requirements or as evidence in legal proceedings.

When computers are used to record samples and/or results, it is often possible to write sub-routines (macros) that check ("mask") the notes for unusual entries and/or check the analytical results for unusual values. When the computer indicates an unusual entry or value, the technician can check the samples again or can run the analyses again to make sure. For how to instigate this checking, please refer to the manual for the software or to the manuals for languages that are commonly used to write macros (such as Basic).

Some routine laboratory procedures can considerably enhance quality control. These standard operating procedures should be recorded in a laboratory guide and carried out on a regular schedule. The following suggestions do not refer to issues of safety.

Reagents should be fresh and uncontaminated. Old reagents or contaminated reagents should be discarded. Reagent bottles should be gently agitated at periodic intervals to insure that the concentration of the reagent is uniform throughout.

Working areas should be neat and orderly. No reagents, glassware or other equipment that is not in current use should be in working areas. Maintain closed containers for discarded filters and other matter that might give off any contaminants. Discard waste daily.

Glassware and other equipment should be washed on a regular basis. If stored glassware is not used within a specific time period, it should be washed again anyway. At regular intervals (from one to six months), all glassware should be acid washed, thoroughly rinsed, and cleanly dried.

Equipment should be clean and in good operating order. Equipment should be calibrated before it is first used, according to the manufacturer's instructions. In general, when working with linear variables, equipment should be calibrated on samples of at least three different ranges; when working with non-linear variables, equipment should be calibrated on samples of at least five different ranges. Equipment should be re-calibrated at regular intervals according to the manufacturer's instructions. Some equipment may need to be calibrated every month. Dissolved oxygen meters can be usefully calibrated daily.

The laboratory should be in a closed room (or building) with air conditioning. If possible, the air conditioning should be "external," with the compressors and filters external to the laboratory area. Air conditioning ducts should be filtered and should be cleaned at least once per year by professional cleaners. If internal, or "window," air conditioning units are used, the filters should be cleaned at least once a month, and replaced whenever necessary. The seals around the unit must be kept in good condition. Malfunctioning units should be repaired immediately.

Computers should be routinely inspected to make sure that the operating system and the program files are not corrupted and are operating properly. The OS and the programs should be re-loaded at periodic intervals. Computers should have virus protection ("fire wall") to catch viruses when they

enter and before they can spread through the computer. It is usually far easier to catch and fix a computer problem before the computer shows symptoms than afterwards.

Table 2. Common tests for the most widely used variables

VARIABLE	METHOD
Water temperature	Ordinary mercury thermometer
Dissolved oxygen	Standard dissolved oxygen meter (such as from Yellow Springs Instrument Company [YSI])
pH	Standard line-powered laboratory pH meter with glass electrode
Total ammonia nitrogen	Phenate method. The salicylate method could be used as an alternative.
Nitrate nitrogen	Diazonium salt method
Total phosphorus	Persulfate digestion with ascorbic acid finish
Total nitrogen	Persulfate digestion with ultraviolet spectrophotometric finish
Chlorophyll a	Acetone extraction with spectrophotometric finish
Total suspended solids	Glass fiber filtration and gravimetry
Biochemical oxygen demand	Standard 5-day test
Salinity	Line-powered conductivity/salinity meter. Alternatives include: chloride concentration in milligrams per liter x 1.90655; or hand-held salinometer.

Alternative tests are available for almost all the variables. Some tests are more sensitive and some have been developed particularly for saline water or for strongly saline water. For alternatives see

Grasshoff, K., M. Ehrhardt and K. Kremling (eds). 1983
 Methods of Seawater Analysis
 Weinheim, Germany: Verlag

Total Quantity of Effluents and Effluent Load

Suppose that a shrimp growing area has 1000 hectares of grow-out ponds. Each hectare has 10,000 meters of surface area. Suppose the average depth of a pond is 1.5 meters. The total volume of water in the ponds is:

$$1,000 \times 10,000 \times 1.5 = 15,000,000 \text{ cubic meters}$$

If the ponds were drained only once per year, and drained completely, then this is the amount of effluent water that shrimp farming would contribute to the local receiving water body.

If more than one crop is grown every year, and the ponds are drained completely for each crop, then the total volume has to be multiplied by the number of crops per year. Suppose that 2.5 crops are grown per year:

$$2.5 \times 15,000,000 = 37,500,000 \text{ cubic meters}$$

The average daily effluent can be found by dividing the above figure by 365. However, since the effluent is not released in small portions daily but in large portions clustered around the end of growing seasons, the effect of the effluent on the receiving water body will be different than if it were released in small increments daily.

If the load in the effluents is the same for every crop, then the total load can be figured by multiplying the total water volume by the load per crop, for any given variable.

Where water exchange is practiced during the crop cycle, the problem becomes a bit more difficult. Say that 30 percent water exchange is practiced during each crop cycle, and then the ponds are drained completely. The exchange could occur in three installments of 10 percent each, or one installment of 30 percent. In either case, the total amount of effluent water has to be increased by the amount of water exchange:

$$37,500,000 + (37,500,000 \times 0.3) = 48,750,000 \text{ cubic meters}$$

However, now we cannot use this figure for water volume directly to calculate the load carried by the effluents into the receiving body of water because it is likely that the effluent load carried by exchange water from the ponds

differs from the effluent load carried by harvest water from the ponds. To figure out the total effluent load, we would need to know the average load in exchange water and the average load in harvest water. We could then multiply the amount of exchange water (37,500,000 cubic meters) by the average load in exchange water, multiply the amount of harvest water (11,250,000 cubic meters) by the average load in harvest water, and add the two together. This calculation is not performed here.