Editorial

Aquabyte has yet to live up to its promises to provide NTAS members with details of better research methods and protocols. We have published a few methodological papers, but most *Aquabyte* issues have featured what might be called awareness or experience papers. In this issue, Dr. James Szyper gives some basic and important advice on planning experiments and writing proposals. Hopefully, this will be the forerunner of many more articles on how to plan, organize and conduct aquaculture research. If you have proven ways of doing things and good experimental protocols, please share them with other NTAS members through *Aquabyte*.

This issue also gives an example of integrated resources management by farmers in Bangladesh and has all our usual features. Early in 1993, we hope to be able to publish a directory of NTAS members and an analysis of the responses to the recent questionnaire. ***R.S.V. Pullin***

A Standard Format for Design and Evaluation of Pond Experiments

JAMES P. SZYPER

The US Agency for International Development (USAID) funds a number of Collaborative Research Support Programs (CRSPs), which conduct research on a variety of food production issues, always with international collaboration among researchers from the host countries and the United States. One research group is called the Pond Dynamics/ Aquaculture (PD/A) CRSP. It aims to improve yields and reliability of animal protein production through increased understanding of pond ecosystems. Researchers from universities and government research stations in four tropical countries (Honduras, the Philippines, Rwanda and Thailand) work with colleagues from six US universities, usually at more than one site in each country. My home institution, the University of Hawaii, participates in projects in Thailand and the Philippines.

The PD/A CRSP prepares Work Plans every two years. They explain each experiment to be conducted. The first three Work Plans specified the same experimental protocols at all sites. Subsequently, the work has included both experiments common to two or more sites, and some site-specific activities. Our need to maintain a well-planned program of high-quality experimental work has led us to evolve a standard format for communicating experimental ideas within the PD/A CRSP group.

Fig. 1 shows a skeleton of what we call the "Preliminary Proposal Format," containing what could be called the "complete" list of information categories or headings. I used quotation marks with "complete" because PD/A CRSP researchers are free to add or subtract from this list as needed in specific cases. This reflects the fact that construction of a preliminary proposal is more complex than the task of filling in a form. These headings, however, are usually both sufficient and necessary for clear description of pond culture experiments.

Label. In this space, we give the activity an identifier, permitting easy subsequent reference.

Title. Writing a good title requires some thought. In two lines or less, the title should characterize the experiment well enough so that a reader who has read the complete description will be clearly reminded of it later by the title alone.

Fig. 1. ***Blank example of the Preliminary Proposal Format.***

Label: **Preliminary Proposal**

Title:
Objectives: 1. To
2. To
Significance:
Experimental Design:
Pond Facilities: **Duration:**
Stocking Rate: ___ **fish/ha or** ___ **fish/pond** **Water Management:**
Other Inputs:
Sampling Plan:
Hypotheses:
Statistical Methods: **Schedule:**

Objective(s). A research objective is an item of information or accomplishment that the proposer wishes to have in hand at the end of the activity. An objective should be stated so that an observer of the project can determine readily, at any time, whether or not the objective has been or can be attained. Attainable objectives should also be of obvious value to a person who is familiar with the overall goals of the PD/A CRSP and its typical activities. The value of the work can be stated specifically in the next section.

Research objectives are distinct from broad program goals; objectives are specific and focused. The statement in the first paragraph of this article which begins, "It (PD/A CRSP) aims to ..." states the program goals, which may be partially attained during various stages of the program and are broader than specific research objectives. We work each year toward improvement of yield and reliability of protein production by acquiring and disseminating new knowledge. Objectives must be neither broad nor open-ended. It would not be acceptable, for example, to state an objective: "To improve yield by applying Treatment X". Whose yield is to be improved, the same persons addressed in the broad goal? Is this one experiment to accomplish that? Is the proposer certain that Treatment X will have significant, entirely positive, and generally applicable effects? A better statement would be, "To examine the effects of Treatment X on pond yields in comparison with controls under the conditions described below."

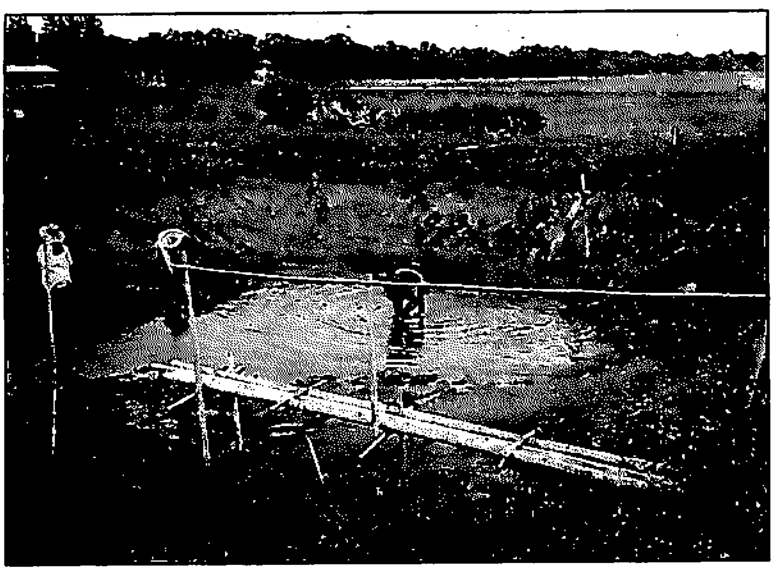
Significance. This section should answer questions such as: "What is the origin and nature of the problem being addressed in this work?"; "How and why were the objectives chosen?"; "How will the information gained serve the broad program goals?"; and "Who will care about or benefit from the results of this work?"

Experimental Design. Here, the proposer is challenged to communicate, in a small space, the essentials of what is to be done. One of the faults most frequently identified in proposals is inadequate description of methods and activities. This section should present the nature of the treatments and the replication scheme. In our format, other aspects of methods are addressed in the succeeding categories but some may be mentioned here also, as needed to complete the overview of the approach.

Pond Facilities. This section specifies the site and the number and size of ponds or other facilities needed. This is of great value for planning. All proposed activities for a site can readily be brought together and a list of required facilities compiled. Such information, along with the Schedule item below, helps to avoid temporal overlap or other conflicts in the use of facilities.

Duration. This is a simple statement of the culture period or duration of other activities.

Stocking Rate(s). Ponds available to our program range in area from 200 to over 2,000 m^2. Expressing stocking rate per unit area permits readers to compare protocols among different facilities; expressing rates on the "per pond" basis facilitates planning for sufficient stock to perform the designed experiment. This program usually uses sex-reversed tilapias and lead time and hatchery facilities must be allocated carefully.



Research pond, Asian Institute of Technology, near Bangkok, Thailand: Planning the experiments carefully is critical for success.

Water Management. Through most of this program and at most sites, water depth has been maintained to the level specified in the design with additions each week and losses, if any, recorded. Recently, however, experiments have begun to address such issues as the effects of water exchange and the functioning of ponds filled by rainfall and allowed to decrease in depth during dry periods.

Other Inputs. Here are specified inputs other than water and fish, namely fertilizers, prepared feeds, or other materials, and the amounts and timing of their application.

Sampling Plan. This section should specify the location and timing of sampling for water, fish, sediments, or any other material or item of information (e.g., recording protocol for weather station or monitoring system), and name the quantities to be determined. PD/A CRSP Work Plans contain summaries of acceptable analytical methods, which make reference to standard works.

Hypotheses. An hypothesis is a suggestion that a particular condition exists or that a particular outcome of an experiment will take place. The hypotheses represent the essential core of the thought behind an experiment; their support or denial is the focus of the activities. They must be chosen so that testing them by means of the experimental design will cause the objectives to be attained.

Hypotheses may predict negative results, in which case they are called "null" hypotheses, for example, "Treatment X will have no significant effect on fish yields under these conditions." The idea of null hypotheses comes to us from the field of statistics, where it is compatible with the logic of probabilistic evaluation of data. Although the null formulation of statements may seem awkward at times, it can help to keep a proposer focused on planning and recording clearly interpretable results. There are some cases of null formulations that sound ridiculous or extremely unlikely, for example, "Hatchery survival of the fish is not significantly affected by feed type or amount, temperature, water exchange, aeration and lighting." Such expressions should be recast in positive terms to make them more informative: "Hatchery survival is significantly favored by the higher protein feeds."

Hypotheses are helpful to planning, communication and evaluation of proposed experiments because they must be phrased, and the work must be planned, so that their clear support or denial is possible with the information to be gathered. If higher protein feeds are properly compared with others, and even in this one experiment fail to favor survival, then the hypothesis is denied because there is one well-established counter example. In general, positive hypotheses can be denied or disproved by a negative experimental result.

Fig. 2. *An example of full-length use of the Preliminary Proposal Format.*

Label: WP 7 Thailand Study 6 **Preliminary Proposal**

Title: Maintenance of Total Alkalinity Levels in Fertilized Ponds for Stability of Phytoplankton Stocks and Photosynthetic Rates

Objectives:
1. To examine the association, if any, between organic fertilizer inputs and stability of total alkalinity concentrations in pond water during typical PD/A CRSP pond fertilization experiments.
2. To examine whether maintenance of nonlimiting total alkalinity favors temporal stability of phytoplankton standing stocks and photosynthetic rates, and growth and yield of fish.

Significance: Stability of phytoplankton stocks and activity is important to successful pond culture in general and to fertilizer-based strategies in particular. Large phytoplankton stocks are often unstable; if low alkalinity limits photosynthesis, instability is likely. Alkalinity can decrease when high stock levels are maintained on inorganic fertilizer; CRSP observers suggest that this is less likely when organic inputs are included. This experiment will compare alkalinity trends under both regimes, and quantify the effect of interim lime addition on downward trends in alkalinity. This experiment begins the Thailand project's approach to understanding carbon cycles in ponds.

Experimental Design: Twelve 0.04-ha ponds will be used, six being fertilized with inorganic fertilizers alone at known effective levels for this site, and six being fertilized with an isonitrogenous (and of similar N/P ratio) combination of chicken manure and inorganic materials. Within each of these two treatments, three ponds will receive no inputs of lime during the trial, while three will receive biweekly additions of lime containing carbon equivalent to the difference between analyses and the inorganic carbon content of the pond when total alkalinity = 100 mg/l as $CaCO_3$.

Pond Facilities: Twelve 0.04-ha earthen ponds **Duration:** 150 days

Stocking Rate: 20,000 fish/ha or 800 fish/pond **Water Management:** Depth to 1.0 m weekly

Other Inputs: Inorganic-only treatment: 28 kg N/ha/week @ N/P = 5.0; urea and TSP.
Organic combination treatment: chicken manure @ 200 kg/ha/week, urea to make up N = 28 kg/ha/week, TSP to approximate N/P = 5.0.

Sampling Plan: Standard protocols except: Analysis of total alkalinity weekly in one depth-integrated sample from each pond. Diel sampling of DO (0600, 0900, 1200, 1500, 1800, 2200, 0600 hours) and pH at three depths (5, 35, 80 cm) every two weeks. Soil pH and base-sat, before filling and after draining of ponds at three locations from each pond.

Hypotheses:
$H_{0.1}$: Nature of fertilizer has no effect on the amount and temporal pattern of total alkalinity under these conditions.
$H_{0.2}$: Total alkalinity is unrelated to phytoplankton stock and photosynthetic rate when concentrations are greater than 50 mg/l as $CaCo_3$.

Statistical Methods: ANOVA, correlation, time series analysis. **Schedule:** 12/93 - 05/94

Statistical Methods. Well-planned experiments yield data of known character, and so it should be possible to name the appropriate major statistical methods to be used in analysis. This provides further indication that the proposer has thought through the entire process. It is recognized, of course, that the specific nature of the results may suggest further examination by other methods.

Schedule. While specified targets are generally important for any complex activity, scheduling is particularly important on the facilities supporting the collaboration of multiple institutions. The beginning and ending months and years are stated here.

An actual preliminary proposal is shown in Fig. 2. The reference to "Standard Protocols" under "Sampling Schedule" indicates the sampling regimes that were standardized during earlier Work Plans. These are too elaborate to permit inclusion in these one-page descriptions. These examples are not necessarily perfect executions of the ideal format, but they represent well-planned descriptions understandable to everyone in the program. If readers of this article find themselves able and wanting to critique these examples, then the editor's implicit hypothesis, that this article might be useful, is supported.

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