PART II. RESEARCH PROJECT INVESTIGATIONS

TOPIC AREA

PRODUCTION SYSTEM DESIGN AND BEST MANAGEMENT ALTERNATIVES

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EXPERIMENTAL POND UNIT ASSESSMENT IN TANZANIA

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Production System Design and Best Management Alternatives/Experiment/16BMA01PU

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Objectives

1. To evaluate ponds in Tanzania for their physical, chemical, and biological characteristics during a grow-out.
2. To compare the effects of fertilization alone, full ration feeding alone, and a combination of fertilization and feeding at 50% satiation on growth performance and production yield of Nile tilapia.

Significance

Aquaculture is considered an option for rural development because it can help to solve problems of protein malnutrition and income poverty and provide employment to rural poor people. In Tanzania, aquaculture is an emerging industry and dominated by pond culture of Nile tilapia (*Oreochromis niloticus*). The industry is dominated by small-scale farmers producing fish for household consumption and for the domestic market. However, fish production in many small-scale ponds is low due to low productivity of the commonly cultured species coupled with poor management. Given the importance of aquaculture in the country, there is a need to improve fish production to complement the declining capture fisheries. Improvement in aquaculture production can enhance food security directly by producing fish for household consumption and by improving the supply and reducing the price of fish in the market (Jahan et al., 2010). Fish consumption has been shown to improve the nutritional status and lower prevalence of malnutrition in rural poor households (Aiga et al., 2009). Fish farming contributes to improvement of wellbeing of poor people indirectly through diversification of sources of income and creation of new employment opportunities. With increased household income from fish farming activities, households are also able to purchase animal based foods with better sources of micronutrients needed by the household members, especially children (Aiga et al., 2009).

Sustainable fish farming depends on the maintenance of healthy pond water environment and the production of sufficient fish food organisms. Among the primary factors limiting the production capacity of a pond is the quantity of available nutrients, which form basic materials for structure and growth of living organisms. Proper pond fertilization and supplementary feeding techniques are used to supply these nutrients in optimal quantities, thereby overcoming natural deficiencies, in order to obtain maximum possible fish yield from a water body. Studies have shown that proper pond fertilization combined with supplementary feeding at 50% satiation level results in higher fish yield and benefit-cost ratio (Wahab et al., 2014; Phanna et al., 2014).

This pond characterization experiment has two goals; the first is to evaluate ponds at each research site for their physical, chemical, and biological characteristics during a grow out, and the second is to compare the effects of fertilization alone, full ration feeding alone, and a combination of fertilization and feeding at 50% satiation on growth performance and production yield of Nile tilapia. The methods for pond characterization are well described in a number of publications, including Egna et al. (1987) and the *Standard Methods for the Examination of Water and Waste water* (multiple versions of this are available; the most recent is APHA et al., 2012). The purpose of this document is to describe a series of measurements that will be carried out for the AquaFish Innovative Lab, and to outline some of the reasons for these measurements.

Physical characteristics

A number of physical characteristics are important in aquaculture, particularly for the completion of quality experiments in ponds. The most obvious ones include pond morphometry, pond depth, evaporation rate, seepage rate, and water temperature.

Morphometry, or the physical shape of the pond, is important because it describes the physical place where experiments occur, and helps to assess water loss and replacement in volume. Usually morphometry is measured from the pond banks, and the actual working pond morphometry then is dependent on how much water is added to the pond. Morphometry can most easily be measured by simply making a number of transects across the pond itself, from the bank top, and measuring the depth at regular intervals (usually some frequency like 10 per transect or every meter) across the transect. In this manner, the surface area and pond volume can be calculated at each depth for the working pond.

Pond depth is a simpler measure that describes the amount of water in a pond at any time. It is usually measured at the deepest point in a pond, which is often either the center or the depth at the drain or monk. The measures of pond depth are usually taken daily, and water is often replaced on a weekly basis to maintain a regular pond depth. Pond depth is also controlled during a rain event by the drain stand pipe or monk boards, which only allow water to reach a certain level before it is discharged. For our work, depth will be measured and water replaced weekly, if necessary. Also, it is important to measure the loss of water from a pond during rainfall events, as this water will carry nutrients and other materials.

Evaporation of water occurs continually from a pond, depending on humidity, temperature, and wind. We can measure evaporation rate simply with an evaporation pan, and relate it to the other physical parameters mentioned above. Typically, evaporation is not measured regularly in ponds, in most cases only 2-3 times per grow out. For our case, that would be at stocking, mid grow out, and harvest.

Seepage is a similar measure that takes into account the water leakage from a pond. It does vary with pond depth, and again it strongly affects water use in aquaculture. Once again, seepage can be measured by evaluating changes in pond depth and those changes expected to occur due to evaporation. We should measure seepage at the same time that we measure evaporation rate, to simplify the calculations.

Finally, water temperature is the last physical parameter to be measured. This is complicated by the fact that ponds are shallow and exposed to sunlight and wind. Therefore, they may warm dramatically during the day, may stratify on calm days, and may mix thoroughly on windy days or at night. Hence, temperature must be measured several times at multiple depths to get relevant information. The existence of data sondes that can monitor temperature and oxygen over time have simplified this process, and it would be most relevant to do continual measurements at about hourly intervals at multiple depths, at least including surface, middepth, and just off the bottom. The measures can provide a good overview of the temperature characteristics influencing the pond, and also can be used to determine rates of primary production in the pond. Finally, because seasonal events also strongly influence temperature, and because it is an important factor influencing animal physiology, we should also measure temperature at three depths on weekly basis.

Chemical Parameters

The chemistry of pond water has dramatic effects on the pond ecosystem as well as the organisms being cultured. It is also an indicator of management methods and their success. While a very large number of variables can be monitored, in our pond experiments we will focus on those most commonly related to production in a pond. These include dissolved oxygen, phosphorus, various forms of nitrogen, pH, alkalinity, and dissolved and suspended solids. For marine or brackish water systems, obviously salinity would be another important factor.

Dissolved oxygen (DO), like temperature, can stratify dramatically in ponds and also changes dramatically over the diel period. Maximum DO occurs during the day in ponds with much primary production, and the minimum occurs at dawn after a long night of respiration alone. Also, if pond water stratifies, then most likely oxygen will stratify even more strongly than temperature. This is particularly true in shallow ponds as most commonly light penetration is limited to the upper 40 cm or so of water, and all primary production occurs in this zone, while in deeper waters, even during the day, limited oxygen may be produced and oxygen levels may decline from the time of first stratification until the pond is mixed again in the evening. Therefore, like temperature, we should measure DO on an hourly basis and at various depths, again most reasonably the surface, midwater, and bottom for ponds of depths around 1 m. The simplest method for this is to use a data sonde that can continually measure and record DO and temperature. If this is not available, then a DO meter can be used and either set up for continual measures or manually used each hour at each depth needed. Since DO is also a very important parameter for survival of animals in ponds, we should also measure it regularly at dawn to evaluate longer term trends. These measures should be done on a weekly basis.

In freshwaters, phosphorus is considered the major limiting nutrient. In such waters, addition of phosphorus in the form of triple super phosphate can result in increased rates of primary and secondary production, and depending on the species present, increased production of the target organisms. While phosphorus is found in several forms, most commonly we have measured the total phosphorus concentration of pond water since the conversion between these forms tends to occur very rapidly. While phosphorus can vary over the day or at depth, it is not so dramatic as DO and temperature. Therefore, we most commonly measure total phosphorus during midday using a mixed water column sample. This mixed sample would include water from all depths of the pond, and is usually collected with a large pipe that can be lowered and sampled to include all depths in the column. The measurement frequency should be weekly.

If phosphorus is the limiting nutrient in pond water, then its addition will increase primary production. However, at some level phosphorus will become available in surplus, and then no further increase in primary production will occur with additional inputs. If regular water quality measurements were being made, this point would be obvious by phosphorus increasing in concentration in the water column. At this point some other nutrient has become limiting, and most likely this will be nitrogen. Addition of nitrogen, in the form of urea, nitrate, and the like can further stimulate primary production. Combined supplementation of nitrogen and phosphorus will then continue to drive up even higher rates of primary production. Pond experiments in Thailand have shown that the optimum rate of fertilization is 4 kg N and 1 kg P per ha per day. At this optimum rate, both nitrogen and phosphorus are input at rates that allow high rates of primary production yet do not result in drastic declines in DO and give high production rates for Nile tilapia.

Nitrogen in pond water varies in form depending on the nitrogen cycle. Depending on the aerobic nature of the pond water, and on pH, some of these forms can be toxic while others are necessary for primary production. For these reasons, all forms of nitrogen are monitored in the pond. Nitrate is the most readily taken up by plankton for photosynthesis, and is often the dominant form of nitrogen in the water. Ammonia (NH_3) is given off as a waste product by aquatic organisms. It can be toxic when it is converted back to ammonium (NH_{4+}) at high water pH. Nitrite (NO_2) is an intermediate form of nitrogen in the nitrogen cycle as it is converted from NH_3 back to NO_3, and can also be toxic to animals at high pH. Finally, total Kjeldahl nitrogen (TKN) is the nitrogen contained dissolved in the water as well as that found in microorganisms in the water column. Dissolved inorganic nitrogen is the sum of NO_2, NH_3, and NO_3. In our pond characterization we will measure all of these forms, including NO_2/ NO_3, NH_3, and TKN. Since nitrogen forms do not show large changes with depth or time of day, they will also be measured weekly using a composite water sample around midday.

pH is the measure of acidity in the water. It is affected by a large number of chemical characteristics, including the water source, the balance of carbon in the water, and other acids or bases in solution. It also can show variations with depth and with time, as it is affected by forms of carbon in the pond and therefore by rate of primary production, at least in waters with moderate to low levels of alkalinity. Therefore, we measure pH hourly at each depth, as it is done with DO and temperature. Data sondes often have pH as an added variable measured, making this a fairly simple measure. Otherwise, manual measurement with a pH meter will be necessary. Since pH influences toxicity of NO_2 and NH_3, we should also measure it weekly on a composite sample.

Alkalinity is a measure of the combination of carbon in the water. It includes carbon in the forms of carbon dioxide, carbonate, and bicarbonate. With more dissolved carbon in the water, usually the water becomes more basic in pH, and therefore the measure of alkalinity is usually done by titrating water to a set level of pH. Highly alkaline waters have lots of carbon for primary production, and therefore are usually limited by phosphorus, nitrogen, or light penetration. However, low alkalinity waters, particularly those below 30 mg/L alkalinity as $CaCO_3$, may become limited in carbon as well. If feed or organic fertilizers are added to pond waters, they can increase alkalinity because they have lots of carbon contained within. However, if just inorganic forms of TSP and NH_3 are used for fertilization, then alkalinity may decline and carbon become limiting to primary production. While we can simply measure alkalinity once a day to get a reasonable idea of the carbon conditions in a pond, if we use the changes in pond water to determine primary production rates, then we should measure alkalinity on a diel basis similar to temperature, DO, and pH. Hence, both weekly and diel measures are appropriate.

The final chemical variable for freshwater systems is a measure of solids. Total dissolved solids include many of the elements listed before, plus others. Suspended solids are those that are in the water column usually due to the source water, but not dissolved. Suspended solids cause turbidity, and can limit primary production by reducing light penetration. While many components of suspended solids will settle out of supply water if it is held in a calm state, some colloids of clay will remain in solution and raise turbidity. In any case, both TSS and TDS should be measured on a weekly basis like phosphorus and nitrogen.

Biological characteristics

Besides the biomass and production of the target organisms in a pond (which we are not measuring in this experiment), other biological characteristics are important. Generally, the interest would be the amount of phytoplankton production in ponds, either by estimating the rate of primary production or the phytoplankton standing crop. While bacteria and other microbes may be very important in pond culture, we have not regularly measured microbial processes in pond waters.

Phytoplankton in the water may be characterized by their species composition, but this is a tedious process and usually not undertaken unless an experimental protocol is particularly interested in the production of certain phytoplankton species. However, total plankton biomass is a variable of interest to most pond culture systems. We can estimate plankton standing crop by measuring light penetration. Since algae in pond water block light penetration, the lower the light penetration, the higher the plankton standing crop. Of course, solids in the water can also influence light penetration, and the best comparisons would be when changes in light penetration are measured over time in a water system. The simplest measure of light penetration is the Secchi disk, which is lowered into the water until it disappears from sight. The amount of light penetration is twice the Secchi disk depth, since for one to see light reflection from the disk, light has to penetrate both down to the disk and back up to the eye. Usually a measure of 2xSDD is considered the compensation point or the depth at which sufficient light penetrates to allow for primary production. Thus, in this case a shallow SDD indicates high concentration of plankton, and a deep SDD indicates less plankton biomass. Since biomass is often related to production, low SDD may also indicate high rates of primary production, although this measure is less directly proportional than the relationship to biomass.

Another measure of plankton biomass is the concentration of chlorophyll in the water column. Since chlorophyll is present in all photosynthesizing algae, it is an indicator of algal biomass. Again, we typically measure chlorophyll-a because it is the dominant form of chlorophyll, and measure it on a weekly basis from combined water samples.

Primary production is a measure of the rate of photosynthesis. Historically, it was measured by the light-dark bottle method, and the increase in DO in a bottle of water with light allowed to penetrate was used as an estimate of net primary productivity (photosynthesis minus respiration in the organisms contained in the bottle), while the decline in DO in the bottle kept in the dark was a measure of respiration only, and the sum of respiration plus NPP gave the gross primary production of the organisms in the bottle. This works well for low productivity, natural waters where it may take several days to measure an increase in the light bottle and a decline in the dark bottle. In highly eutrophic aquaculture ponds, the high amounts of material added result in high rates of respiration, and dark bottle DO often declines to near zero in a matter of hours. Similarly, since light penetration and primary production are related to depth and stratification in ponds, a number of bottles at different depths would be necessary to reasonably approximate whole pond production. Several studies have shown that the light-dark bottle method is not suitable for estimating primary production in aquaculture ponds.

A second method which is preferable as an estimate of primary production in aquaculture ponds is the whole pond method. This method uses the increases in DO during the day and the declines at night to approximate photosynthesis and respiration in the pond. Of course, diffusion at the pond surface may also influence DO levels, so it may be necessary to correct for diffusion in the estimate. Once again, since the values of DO, pH, alkalinity, and temperature are being collected at 3 depths and on a regular basis over the diel period; we can use these to calculate the net primary productivity, respiration, and gross primary productivity of the whole pond system. Templates for these calculations are available in several CRSP documents. For a reasonable estimate of whole pond production, measurements of DO, temperature, pH, and alkalinity should be taken at dawn, midday, dusk, midnight, and the next dawn.

Summary

This document lists the main water quality variables to be monitored in pond aquaculture, and the reasons for the number and frequency of those measurements. A summary table below lists the variables again and their metrics. Methods for the measurement of each parameter are described in Egna et al., (1987) and are partly based on APHA et al., (2012).

Quantified Anticipated Benefits

The completion of a pond characterization experiment in Tanzania will provide a complete understanding of the strengths and weaknesses of IAAS for further experiments. Through this understanding improvements in the infrastructure and training of project personnel could be done if necessary to fulfill future research. In addition, a better understanding of the physical, chemical, and biological characteristics of ponds in Tanzania will aid culturists in determining better methods of feeding, fertilizing, and managing water quality. This could lead to increased profits, the supply of high quality fish protein to communities with limited food resources, and the overall growth of the aquaculture sector. The expected deliverables are as follows:

1. Better management strategies for pond fertilization and fish feeding determined and promoted;
2. Data for physical, chemical, and biological characteristics of ponds established for future AquaFish innovative lab experiments;
3. At least 40 fish farmers trained through training workshops and adopt better pond fertilization and fish feeding strategies;
4. At least five MSc students trained on pond water quality management strategies.

Research Design and Activity Plan

The experiment will be conducted in 12 earthen ponds, each with the size of 100 m^2. There will be three treatments and each treatment replicated four times. Treatment 1 (T_1) will be the control involving four ponds receiving fertilization alone. Treatment 2 (T_2) will involve four ponds with feed applied *ad libitum*, (only feeding with no fertilizer application). Treatment 3 (T_3) will involve four ponds with a combination of both feeding and fertilizer application, feeding will be at half satiation (fertilization plus supplementary feeding at 50% satiation level). The treatments will be randomly allocated to the experimental ponds in a completely randomized design.

Location

The study will be conducted at the Aquaculture Research Facility, Department of Animal Science and Production, Sokoine University of Agriculture.

Methods

Before the start of the experiment water will be drained from all ponds. The ponds will be left empty for a period of 14 days to allow them to dry. After drying, agricultural limestone ($CaCO_3$) will be applied to the pond bottom and the slopes of the dykes at a rate of 100 g/m^2 and after seven days the ponds will be filled with water to a depth of 1 m. All ponds will be stocked with sex-reversed Nile tilapia (*Oreochromis niloticus*) at a stocking density of 2 $fish/m^2$. Fertilization will commence one week before stocking. Fertilization will be at 4 kg N and 1 kg P per hectare daily, using urea and Diammonium phosphate (DAP), and will be applied once a week. Feeding will be done twice daily, in the morning (10.00 am) and in the evening (16.00 pm). Locally available feeds will be used and the diet will comprise of wheat bran (50%), fish meal (25%), cotton seed cake (10%), sunflower seed cake (11%) and maize meal (4%). The diet will be formulated to contain 30% CP.

Pond water level will be maintained at a depth of 1 m by topping up weekly to replace losses due to seepage and evaporation. Ponds will be managed for 120 days, and then harvested. Weekly measurements of water quality parameters as described above and also in Table 1 will be done in mid-week from water collected at the center of the pond. Diel measurements will be made biweekly up to the end of the experiment using the methods described above. Temperature in ^{0}C and dissolved oxygen mg l^{-1} will be measured by using YSI 55 dissolved oxygen meter (model 55, Yellow Spring Instrument Co. Ohio, USA). Water pH will be measured using a pH meter (Mardel 5, R029B-MARDEL, USA). Total alkalinity, nitrate-nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), ammonia-nitrogen (NH_3-N), total dissolved solid (TDS), conductivity, and phosphate-phosphorus (PO_4-P) will be determined using HACH Kit (DR - 4000, a direct reading spectrophotometer). The transparency of water will be measured using a secchi disc of 20 cm diameter. Chlorophyll-a will be determined spectrophotometrically following GF/C glass fiber filtration and acetone extraction procedure.

Body weight and length of individual fish in each pond will be weighed at the start of the experiment to determine initial weight and length, respectively, and then every two weeks up to the end of the experiment. Body weight will be weighed in gram using a sensitive weighing balance and body length will be measured in cm using a measuring ruler. Death of fish will be recorded every day. At the end of the experiment, fish will be harvested by repeated netting using a seine net and then by de-watering the ponds using low-lift pump. Body weight and length of harvested fish will be weighed to determine final body weight and length, respectively.

Data including yield, growth rate, and survival will be computed for fish from all treatments, and comparisons made using analysis of variance (ANOVA). Differences in water quality between treatments and over time will be tested using ANOVA. In addition to chemical concentrations, diel measurements will be used to determine stratification in the ponds and primary productions rates. These will also be compared among treatments using ANOVA.

Table 1. Physical, chemical, and biological characteristics to be sampled during pond characterization.

Variable	Daily Frequency	How often	Type of sample
Pond morphometry	-	Once	
Pond depth	Once	Daily	One
Evaporation	Once	biweekly	One
Seepage	Once	biweekly	One
Temperature	Diel measures	biweekly	3 depths
Dissolved oxygen	Diel measures	biweekly	3 depths
pH	Diel	biweekly	3 depths
Alkalinity	Diel	biweekly	3 depths
Water depth	Once	Weekly	Whole pond
Temperature	Once	Weekly	3 depths
DO	Once	Weekly at dawn	Composite sample
pH	Once	Weekly	Composite sample
Alkalinity	Once	Weekly	Composite sample
Total phosphorus	Once	Weekly	Composite sample
Total Kjeldahl nitrogen	Once	Weekly	Composite sample
Ammonia	Once	Weekly	Composite sample
NO3/NO2	Once	Weekly	Composite sample
Secchi disk depth	Once	Weekly	Whole pond
Chlorophyll-a	Once	Weekly	Composite sample

Trainings and Deliverables

- Five MSc students will be involved in this research project as one way of long-term training and capacity building on assessment of pond dynamics.
- One workshop will be conducted for fish farmers and Village Extension Officers to train them on water quality management. The target is to attract 40 participants from at least four villages.

- The project will be gender sensitive and it will ensure that 50% and 10% of the farmers attending the training are females and youth, respectively.

Schedule

	2016						2017											
	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
Activity 1.1: Draining of water and drying of experimental ponds	▨																	
Activity 1.2: Liming of experimental ponds	▨																	
Activity 1.3: Filling water and fertilization of experimental ponds	▨																	
Activity 1.4: Stocking of fingerlings	▨																	
Activity 1.5: Weekly fertilization of experimental ponds	▨	▨	▨	▨	▨	▨												
Activity 1.6: Daily feeding of fish	▨	▨	▨	▨	▨	▨												
Activity 1.7: Data collection by measuring water quality parameters	▨	▨	▨	▨	▨	▨												
Activity 1.8: Data collection by measuring fish body weight and length	▨	▨	▨	▨	▨	▨												
Activity 1.9: Fish harvesting and determination of production yield						▨												
Activity 1.10: Data analysis and report writing						▨	▨											
Activity 1.11: Training workshop								▨	▨	▨		▨						▨
FINAL REPORT WRITING							▨					▨						▨