

## AN ABSTRACT OF THE THESIS OF

Luis Franco for the degree of Master of Science  
in Fisheries Science presented on September 27, 1991.

Title: Nile Tilapia (*Oreochromis niloticus*) Production in  
Tropical Microcosms Fertilized with Rabbit Excreta.

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Abstract approved by:

  
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This investigation explored the use of microcosms as a tool for studying the dynamics of tropical aquaculture ponds. The potential use of rabbit excreta as a pond fertilizer in integrated farming systems was also investigated.

Twelve insulated fiber glass tanks were utilized as microcosms to simulate earthen ponds. Seven hand-sexed Nile Tilapia *O. niloticus* were stocked per tank, and microcosm performance was observed for a 90-day experimental period. Three rabbit excreta loading rates corresponding to 50 and 75 kg/10,000 m<sup>3</sup>/day, and a continuously adjusted manure loading rate were assessed. The fertilizer treatments were compared to a control treatment where fish were fed on a prepared food. Water quality variables and fish performance were regularly monitored. Nitrogen and phosphorous content of rabbit excreta were measured.

The dynamics of the microcosms were similar to warm water earthen

ponds with respect to physical and chemical characteristics. Statistical differences were detected between control and fertilized treatments in relation to dissolved oxygen levels, net primary productivity, total alkalinity, total ammonia and orthophosphate levels. Primary productivity was influenced more by light intensity and penetration than by nutrient limitation. Rabbit excreta overloading was observed in the 75 kg treatment. Fish growth was greatest in the control treatment, but it was not statistically different from the continuously adjusted fertilizer treatment (Pondclass) (0.0065 and 0.0056, respectively). Low daily fish gains were observed in the 50 and 75 kg treatments. Low dissolved oxygen and high total ammonia concentrations resulted in low weight gains and condition indices of fish in the 50 and 75 kg treatments. Extrapolated fish yields corresponded to 6,205, 4,563, 3,686, 4,869 kg/ha/year for control, 50 kg, 75 kg and Pondclass treatments, respectively. The observed yields are comparable with field experiences in real ponds. The continuously adjusted treatment showed the lowest manure conversion ratio (3.85) in the fertilized treatments.

The nitrogen content of rabbit excreta varied according to rabbit size, presence or absence of urine plus water waste, and food droppings. Urine plus water waste provided 28 % of the total nitrogen content in rabbit excreta, whereas food droppings provided 12 %. Rabbit urine may play an important role in aquacultural systems because it contains a large fraction of nitrogen in inorganic forms which are readily utilized for algal growth. Other rabbit excreta characteristics such as buoyancy may be advantageous in aquacultural systems. Rabbit excreta is a potentially rich source of fertilizer for use in fish ponds.

Nile Tilapia (Oreochromis niloticus) Production  
in Tropical Microcosms Fertilized with Rabbit Excreta.

by

Luis Franco

A THESIS

submitted to

Oregon State University

in partial fulfillment of  
the requirement for the  
degree of

Master of Science

Completed September 27, 1991.

Commencement June 1992.

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## **ACKNOWLEDGEMENTS**

I am deeply thanked to my parents, Augusto A. Franco and Ana M. de Franco, brothers and sister Augusto, Byron, Sergio, Saul and Ana, for their constant encouragement to achieve new goals.

I acknowledge the LASPAU-Fulbright Program and Universidad de San Carlos de Guatemala, for their financial support.

I gratefully thank my adviser professors, Dr. James E. Lannan, P.R. Cheeke, and especially to M.S. Wayne K. Seim for their constant encouragement and guidance in this study.

Many people were involved somehow or another in the technical support in this study. Dr. N. Patton and Anne Ayers from the Oregon State University Rabbitry Research Center to whom I wish to express my sincere appreciation. Similarly, I appreciate to the Oak Creek Biology Laboratory co-workers for their friendship and welcoming moments.

To my friends and colleagues, Andy Snow, Shree Nath, Enrique Roldan, Nobuya Suzuki, Anne Ayers, Maria Diaz, I wish to express my deep gratitude for sharing with me the student's everyday life, and their friendship. To Kristin A. Tehrani, for her understanding and never-ending support which encouraged me to achieve this new professional goal in my life, I am deeply thanked.

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**Nile Tilapia (Oreochromis niloticus) Production in  
Tropical Microcosms Fertilized with Rabbit Excreta.**

**INTRODUCTION**

Aquaculture and small-animal farming systems and their integration in the developing world show promising results in production of animal-origin protein (Buck, 1978; Huss, 1982; Lebas, 1983; Lukefahr and Goldman, 1987; Rastogi, 1987). Further benefits can come from diversification of production technologies and integration of all components of the farming system (Buck, 1978; Porras, 1981; Hopkins and Cruz, 1982; Devaraj, 1987; Gonzalez et al., 1987).

One aspect of farm system integration is expanded use of animal manures. Indeed, manures play an increasingly important role in subsistence-family farming systems. Because manures are by-products which can be recycled naturally, they are widely utilized as organic fertilizers to augment fish production in ponds. The Chinese have utilized manures to fertilize fish ponds for centuries (Tang, 1970; Lin, 1982). Similar aquacultural use of manure fertilizers has been long practiced in Central European countries (Wojnarovich, 1979) , Israel (Schroeder, 1974; Rappaport et al., 1977), and the Philippines (Hopkins and Cruz, 1982). More recently, Latin American countries have initiated

research and transference of this technology (Porrás, 1981; Porrás, 1984; Delgado, 1985; Gonzalez, et al., 1987; Guillen, 1989).

There are two methods of applying animal manures as fertilizers for fish ponds. Barash et al. (1982) characterized these as follows:

- 1) Semi-integrated systems, in which animals are not kept close to the ponds, so manures must be manually applied.
- 2) Completely-integrated systems, in which animals are kept next to or even penned over, the fish ponds. In these systems, wastes may be either voided directly into the pond by the animals, or are delivered by disposal channel as the animals void them.

Completely-integrated animal husbandry-fish farming systems have several ecological, economic and social advantages over less-integrated systems. In terms of overall resource utilization, the nutrients and energy contained in animal wastes, including that in feces, urine and feed droppings, may be recycled by converting them into pond micro-organisms that, in turn, are consumed by fish (Pearce, 1979; Barash, et al. 1982). The resultant fish flesh then becomes an important product not only for enhancing the nutritional quality of poor people's diets, but also for generating income (Buck et al., 1978; Wohlfarth and Schroeder, 1979; Woyanovich, 1979).

Irrespective of the potential practical benefits to be obtained from integrated systems in aquaculture, there are at least two major

constraints to their wider application in less developed countries.

First, the present methods of applying animal wastes to fish ponds are not reliable. The effects of manure application on fish yields are variable, ranging from highly beneficial to highly detrimental.

Additional research is needed to advance human understanding of the dynamics of fertilized ponds. Second, although most modern integrated farms utilize large livestock (cattle and swine) or poultry, the former are not suited for many subsistence farming opportunities in less developed countries. It has been suggested that small mammals, especially rabbits, would be better suited for many integrated subsistence farms. More experience and research is required to further understand the potential advantages and disadvantages of integration rabbits into sustainable subsistence farming systems.

### **Pond Dynamics**

Animal manures provide a rich source of inorganic and organic nutrients which are naturally recycled by micro-organisms into new organic compounds or released as nutrients. However, animal manures differ each to other in the bioavailability of nutrients (Wohlfarth and Schroeder, 1979). The release of inorganic nutrients in water may enhance or deter development of the biological community in a pond, especially organisms that constitute potential food items for cultured fish.

Primary productivity (incorporation of inorganic carbon into organic material through the photosynthetic process) is known to be correlated with yields of many fish species (Batterson, et al. 1988, Knud-Hansen, et al. 1991). Primary productivity in aquacultural systems

depends on inorganic nutrient inputs, especially carbon, nitrogen and phosphorous (Schroeder, 1978; 1987, Boyd, 1979; Edwards, 1980; Knud-Hansen et al. 1991). Thus, in addition to sunlight, inorganic nitrogen, phosphorous and carbon are considered to be the main limiting factors of algal primary production in closed systems (Lannan et al. 1991; Knud-Hansen 1991).

Organic matter also plays an important role in manured ponds. The decay of organic matter in the pond consumes dissolved oxygen and can cause deleterious effects on fish yield (Schroeder, 1974; Schroeder, 1978; Stickney, et al. 1979; Wohlfarth and Schroeder, 1979; Edwards, 1980). Additionally, the high ion-exchange capacity of organic matter can have a beneficial effect in helping to clear suspended-soil turbidity in pond water (Boyd, 1979). Finally, organic matter stimulates or enhances detrital processes. The role of bacteria as a food source to fish is not completely understood, but Schroeder (1979) and Kirchman and Kucklow (1987) suggested that detrital-bound bacteria may be an important source of protein and amino acids for fish.

In addition to differences associated with the types of manures used, yields in fertilized fish ponds vary according to species and stocking density. In general, ponds under intensively-manured conditions have produced between 9 and 50 Kg/ha/day of fish meat. Maximal yields are obtained through polyculture or multi-species systems (Moav et al. 1977; Buck et al. 1978). Many fish species have proved to be highly productive under manured-pond conditions. Of these, tilapia species are some of the most widely used around the world. Nile Tilapia (Oreochromis niloticus) has been successfully introduced and

grown in developing tropical countries under either monoculture or polyculture production systems (Moav, et al. 1977; Stickney and Hesby, 1977; Rappaport, et al. 1977; Batterson, et al. 1988; Egna et al. 1991). O. niloticus is a hardy species able to survive extreme conditions of water quality, especially at low dissolved oxygen and high un-ionized ammonia concentrations (Stickney and Hesby, 1977; Stickney, et al. 1979; Burns and Stickney, 1980; Lovshin and Pretto, 1983; Msiska and Cantrell, 1985; Gonzalez 1987). Bowen (1982) reported that O. niloticus feeds at the base of the food chain with phytoplankton as their chief food source; however, as filter-feeders, they are also able to ingest most filterable nutritive substances crossing through the gills, including planktonic algae, periplankton, rotifers, copepods, insect larvae and detritus (Edwards, 1980; Fernando, 1983; Batterson et al. 1988).

O. niloticus stocking densities vary in concordance with the system's production objective and whether the system is operated as a mono-culture or poly-culture. Stocking densities of 10,000 fish/ha are frequently used for mono-culture systems wherein average growth rate ranging from 0.50 to 1.8 g/fish/day have been reported (Rappaport, 1977; Hopkins and Cruz, 1982; Batterson et al. 1988).

### **Microcosms: A tool for Studying Pond Dynamics**

Conducting statistically valid, replicated experiments in pond dynamics requires numerous ponds with similar characteristics. The lack of suitable experimental ponds has constrained research programs in this field. The use of ecological micro-systems or microcosms (Warren and Davis, 1971) may be a plausible alternative that could accelerate

research programs in this field. However, this potential opportunity has not been widely appreciated or explored. Research done under laboratory conditions has undoubtedly helped investigators to interpret what happens in nature regarding fish development. Warren and Davis (1971) summarized advantages and disadvantages of using laboratory streams and closed systems as preliminary or alternative studies to whole-pond investigations. Simplicity within spatial limits, and ease of control, manipulation and measurement, are some of the characteristics of these systems. According to Warren and Davis, aquatic ecological micro-systems (microcosms) may be employed to interpret certain phenomena related to fish physiological and behavioral ecology, especially toxicity-stress related problems. However, they also caution that reduced ecological complexity of man-built systems, changes in the behavior of animals living in constrained conditions, and non-equal community representation in micro-systems may lead to misinterpretation of results.

A pioneer study relating primary productivity to inorganic nutrients using microcosms was done by McConnell (1962). His microcosm tended to become supersaturated in oxygen, which then appeared to cause stunting in phytoplankton, reduction in primary production and other harmful effects on the cultured fish. He recommended the use of artificial aeration to promote gas exchange in microcosms-based studies in the hopes of avoiding these and other confounding effects.

Evaluation of fish production in concrete tanks has also been reported. Generally, the results differed from those in the field to a variable extent (Shell, 1966; Delgado, 1985; Msiska and Cantrell, 1985).

This potential for ambiguity in results points toward the necessity for careful experimental design, thorough data collection and cautious analyses of results in studies using aquatic microcosms.

#### **Appropriate integration: Small animals for small farms**

Economic, social and political problems limit the development and advance of applied integrated systems technology in developing countries. Huss (1982) mentions that poverty and small land holdings are common problems in those countries, and usually only time-tested traditional agricultural methods are used. Subsistence production systems are characterized by low material and high labor inputs, with consequent low, but reliable outputs (Owen, 1976; Huss, 1982; Devaraj, 1987). Several programs already exist in developing countries that specifically emphasize the principle of "small-animals for small-farms" (Huss, 1982; Lukefahr, 1988).

The domestic rabbit (Oryctolagus cuniculus) has high potential for culture in developing countries (Owen, 1976, Cheeke, 1979, 1986; Lebas, 1983; Rastogi, 1987; Lukefahr, 1988). Rabbits do not compete with humans for food resources, mainly because they are able to efficiently digest and assimilate high fiber content forages which humans rarely utilize.

Minimal land availability in developing countries makes rabbit husbandry advantageous over other animals. Many rabbits can be raised in a small area. For example, Lebas (1983) stresses that a doe can produce 40 kits per year per square meter cage. This potential density notwithstanding, Owen (1976) suggests a number of 16 kits per doe per

year as a realistic productivity for subsistence-farm conditions.

Rabbit husbandry offers several other advantages. Small-bodied animals such as rabbits simplify handling and harvesting. Each additional animal added to a husbandry system requires only small increased in feed. Additionally, poor families in developing countries may kill and eat a harvested rabbit at once, avoiding the need for meat conservation methods such as refrigeration (Cheeke, 1979, 1986; Rastogi, 1987; Lukefahr, 1988). Finally, rabbits are extremely fast-growing animals, with only 3 to 4 months being necessary to obtain a marketable-size animal (Huss, 1982; Lebas, 1983; Rajadevan and Ravindran, 1986).

Highly prolific reproduction and genetic diversity are also characteristics of rabbits which contribute to their desirability for husbandry in developing countries. Does may be bred a day after parity (Lebas, 1983). High reproduction rate facilitates genetic improvement programs, and wide genetic diversity in available stocks constitutes a useful resource for matching phenotypes to local environmental conditions (Lebas, 1983; Rastogi, 1987). Finally, rabbit production in subsistence systems does not require either expensive equipment or construction materials. The rabbit husbandry system may be installed using locally-available materials, rather than imported materials or those requiring expenditures of scarce hard currency (Owen, 1976; Cheeke, 1979; Lebas, 1983; Lukefahr and Goldman, 1987; Lukefahr, 1988)

Subtropical and tropical conditions exert adverse effects on rabbit production. High temperatures (above 30 C) reduce food intake (Owen, 1976; Cheeke, 1979, 1986; Lebas, 1983; Rastogi, 1987). Fertility problems in males may also be associated with high temperatures (Lebas,

1983).

Forages are the principal food source for rabbits in the tropics. Ekpenyong (1986), Raharjo et al. (1986), and Raharjo et al. (1988), evaluated the nutritional value of forages such as legumes, grasses, and some agricultural by-products grown in different tropical countries. They concluded that woody legumes are valuable resources as feedstuffs for rabbit production. Grasses presented high fiber content and their rough consistency affected feed intake, resulting in poor general performance. Conversely, non-woody legumes with low fiber content often led to enteric distress. Some tropical forages may contain natural toxic substances but Cheeke and Shull (1985) showed that the small herbivores have the digestive capacity to detoxify some of these substances. Diseases and general inexperience in rabbit management also limited the success of rabbit production in some tropical countries (Owen, 1976; Cheeke, 1979, Lukefahr, 1988).

Rabbit meat is the principal product of rabbit production systems. Rabbit meat is lower in fat and sodium, giving it advantages over other meats traditionally consumed by humans (Owen, 1976; Lukefahr, 1988). In addition to meat, other products with economic potential from rabbit husbandry systems include rabbit pelts (Huss, 1982; Cheeke, 1986; Mahadevaswamy and Venkataraman, 1988) and rabbit manure for use as organic fertilizer in fish ponds and as raw matter for biodigesters (Aubart and Bully, 1984; Rastogi, 1987; Mahadevaswamy and Venkataraman, 1988).

A chemical analysis of rabbit manure was presented by Chawan et al. (1979). From the aquacultural point of view, rabbit manure is a

rich source of nitrogen, phosphorus, calcium, trace elements, fat, cellulose and lignin (Chawan et al. 1979; Mahadevaswamy and Venkataraman, 1987). Gilbertson and Clemens's research (1986) showed that rabbit urine may provide a greater fraction of salt content that did fecal matter alone (prepared feed was utilized in the experiment).

## OBJECTIVES

In view of the opportunities, constraints, and research needs summarized above, the objectives of this study are:

1. To evaluate the use of microcosms as a tool for investigating the dynamics of fertilized tropical ponds, and
2. To determine the suitability and characteristics of rabbit excreta for use as an organic fertilizer for culture of Nile Tilapia.

## MATERIALS AND METHODS

### Microcosm characteristics

Twelve insulated fiber glass tanks were used as microcosms to simulate earthen ponds. The tanks were housed at the Oak Creek Laboratory of Biology, near Corvallis, Oregon (USA). The tanks were 1.12 m long, 1.25 m wide, and 0.48 m deep. Each tank water capacity was 0.65 m<sup>3</sup>.

Light was provided through a set of metal halide lamps suspended over the tanks. Light intensity was measured with a LI 185B Quantum/Radiometer/Photometer device (LI-COR Inc./LI-COR Ltd. Nebraska). The light intensity was adjusted to 100 watts/sec/m<sup>2</sup> (24 cal/sec/m<sup>2</sup>) at the water surface. A 12-hour timer-controlled photoperiod was utilized throughout the experiment.

The water temperature in the tanks was maintained at using one 250 watt submersible water heater per tank. Wind was simulated with a fan which served to constantly ventilate and create surface water turbulence. Wind speed was measured using a Sensitive Anemometer (3034/AC, C. F. Casella & Co.Ltd, London). Moderately hard stream water was utilized to replenish the water loss from tanks by evaporation.

A layer of soil approximately 5 cm-thick mixed with rabbit manure was supplied to each tank to promote microbial activity as occurs in earthen ponds. The tanks were maintained partially full of water for 30 days, and completely full for 15 additional days prior to beginning the experiment. This procedure enhanced plankton development and stabilized water quality variables. Calibration of heating and light conditions was

accomplished during this period.

### Experimental Design and Fish Management Procedure

Microcosm performance was evaluated for a 90 day period. Three rabbit excreta loading rates were evaluated and compared to a control group. Four treatments with three replicates each allocated in a completely randomized design were assessed. The treatments corresponded to two constant loading rates, 50 and 75 kg fertilizer/ 10,000 m<sup>3</sup> /day (dry weight), and a third treatment managed according to the 10-day period updated output calculated by Pondclass, a pond management computer program developed by Lannan et al. (1991). The Pondclass outputs are based on availability of inorganic carbon, nitrogen and phosphorous, and dissolved oxygen measured at dawn. Microcosms receiving fresh rabbit excreta were fertilized once a day on a daily basis, except on week 8 when they received the weekly loading rate in five applications. Fish in the control treatment were fed on a 35 %-protein trout floating-pellet feed daily, corresponding to 2 %/ day of the average estimate fish biomass.

Nile tilapia O. niloticus fry of the Ivory Coast strain were obtained from Auburn University. Young fish were maintained in aquaria and received formulated fish feeds for 7 months before stocking at a weight of about 35 g each. At this size their sex could be determined by examination.

Seven hand-sexed O. niloticus (5 males and 2 females), each weighing  $34.9 \pm 5.0$  ( $\pm$  = SD) g, were stocked per tank. This density is equivalent to 50,000 fish/ha. Four fish per tank were collected by seining and weighed and measured (standard length) monthly. Progeny

production was determined by counting all the fry and embryos at each sampling moment or when fry were seen swimming near the surface. All collected fry were removed from the tanks to avoid competition for food resources with adults. Average individual weight of the fry was estimated from group-weighed samples.

An average condition factor (weight/length relationship) as used by Stickney and Hesby (1977), was estimated at stocking as well as at each sampling period for each microcosm. An average percentage daily gain ( $APDG = \ln W_1 - \ln W_2 / t - t_0$ ) equation as described by Winberg (1971) was used to estimate fish growth rate. Average food and manure conversion ratios during the 90 day experimental period were calculated as the total amount of supplemental feed and rabbit excreta applied divided by the fish yield (both on wet weight basis)(Wohlfarth, 1978).

#### **Water Quality Analyses:**

Dissolved oxygen (DO) concentration (mg/l) was measured using a dissolved oxygen meter (YSI model 58; Yellow Spring Instruments Co., Inc., Ohio). DO and temperature measurements at 07:00 h (before lights were turned on), at 14:30 hrs (afternoon), and 20:30 hrs (one and one half hours lights turned off) were measured on 23 sampling days throughout the experimental period. Temperature was measured at the bottom of the tanks and the water surface. The pH value was determined twice a day using a pH meter.

Total ammonia concentration was measured with the nesslerization method (APHA, 1985). Alkalinity and orthophosphate were measured using water analysis kits (Models FF-1A, and PO-19A, respectively, Hach Company, Loveland, Colorado). These water quality variables were

measured every 10 days.

Gross and net primary productivity were calculated using a modified diurnal curve method (McConnell, 1962). This method assumes that the respiration rate during daylight and darkness are equal, although it is recognized that respiration rate during daytime hours may be higher than respiration during dark hours. Carbon fixation was estimated from production by using the molecular weight ratio carbon to oxygen, 0.375 (Boyd, 1979).

Chlorophyll a at the end of the experiment was measured spectrophotometrically according to Strickland and Parsons (1968).

#### **Rabbit Management Procedure**

Fresh rabbit excreta from New Zealand rabbits was collected at the Oregon State University Rabbitry Research Center. A doe and its litter, and grower-finisher rabbit groups were utilized as sources of excreta for the microcosms. It was assumed that does and their litters, and grower-finisher rabbits would adequately represent more than 60 % of the animals at a typical rabbitry (Lebas, 1983). The animals used as excreta sources were randomly selected and replaced frequently by other similar animals. Aluminum trays placed underneath the conventional wire rabbit production cages served to collect droppings. Excreta included feces, urine, water waste, and food droppings. Excreta was collected once a day unless temperature exceeded 22 C; in this case excreta was collected twice a day in order to minimize nitrogen losses by volatilization. Dry matter content was analyzed once a week from a refrigerated pooled sample following procedures of AOAC (1984). Dry

samples were ground and stored under refrigeration for later total nitrogen and phosphorous analyses. Total phosphorous was analyzed by the persulfate method (APHA, 1985).

A second separate experiment was carried out to determine the relationship between rabbit size and rabbit excreta output. Twelve rabbits were randomly selected, divided into three four-rabbit groups according to size bases were confined in twelve metabolism cages. Additionally, a doe and its litter were placed in a well-conditioned conventional cage for 3-day adaptation and 7-day collecting periods. The rabbit groups are described as follows: 1) A doe and 9 kits; 2) Weanling rabbits (weight < 1 kg); 3) Grower-finisher rabbits (weight between 1.2 and 2.0 kg.), 4) Adults. Rabbits were fed on OSU formulated diets on an ad libitum basis. The amount of total excreta (dry weight) per rabbit size group was expressed as a percentage of the total live weight (TLW). Weights of fecal matter, urine plus water wastes, were measured daily. Samples of rabbit excreta samples without the food waste fraction was collected after a 24-hour fasting period. Samples were analyzed for dry matter according to AOAC (1984). Total nitrogen in urine was determined by the Kjeldahl nitrogen method (AOAC, 1984). Fecal nitrogen was determined by combustion in an automated CHN analyzer (MSI Analytical Lab, University of California, Santa Barbara).

#### **Statistical Methods**

Analysis of variance, multiple mean comparisons, regression and other statistical analyses presented in this project were accomplished on a personal computer using STATGRAPHICS (Statistical Graphics Corporation, STSC, USA, 1987).

## RESULTS

To be consistent with the objectives of this study, observations on microcosm dynamics and rabbit excreta as a potential fertilizer for aquaculture ponds will be described in separate sections.

### Microcosm Dynamics

The biological productivity of fertilized fish ponds, including fish yields, is related to the dynamic chemical, physical and biological processes that occur in the ponds. One approach to evaluating the suitability of microcosms as a tool for studying pond dynamics is to observe patterns in chemical, physical and biological processes that influence the performance of the microcosms, and compare these patterns with observations from real ponds. Variables that are useful indicators of the kind and extent of pond processes that are occurring at a given time include temperature, dissolved oxygen concentrations, and various water chemistry variables such as water pH, alkalinity, and dissolved inorganic nitrogen and phosphorus concentrations. The patterns of changes in these variables provide, in turn, useful inferences about primary (plant) production and fish production.

### Temperature

Mean daily morning and afternoon water temperatures were statistically higher in fertilized compared to control treatments ( $P < 0.05$ ) (Table 1). However, mean temperatures differed by only 0.6 C or less between treatments. A daily temperature increase of up to of 4 C,

relative to temperature at dawn was commonly recorded in the 50 and 75 kg treatments by noon. Temperatures in the Pondclass treatment were similar to those recorded for the control treatment. Temperature variations among microcosms within the same treatment was attributed to water heater function. Deposits of manure and detritus which may have influenced thermostat function were frequently removed from heater surfaces.

No thermal stratification was observed in microcosms during the 90-day experimental period, although there was DO stratification, especially by afternoon when super-saturation was recorded in the surface water layer but not from the bottom.

Wind speed inside the lab averaged 0.80 and 1.1 m/sec during day and dark times, respectively.

### Dissolved oxygen

Mean dissolved oxygen concentrations were statistically different ( $P < 0.05$ ) among diel sampling times (Table 1). Mean DO concentrations at dawn for microcosms fertilized with rabbit excreta were lower than for the control treatment. The dissolved oxygen was less than 100 percent saturation in all microcosms during morning measurements. Many DO values below 2 mg/l were tabulated from samples of bottom water from microcosms in the 75 kg treatment. As the day progressed, DO concentrations increased, with maximum DO values recorded at around 14:30 hrs. (Table 1). Afternoon super-saturation above 140 % occurred in surface water layers, especially in microcosms fertilized with rabbit excreta. Weekly, mean DO concentrations for bottom and surface waters

for each treatment indicate that relatively stable levels existed after an initial decline in all tanks over the first 6-8 weeks of the experiment (Figures 10-13, Appendix).

Fish introduction caused changes in microcosm behavior, especially on the dissolved oxygen diurnal cycle. McConnell (1962) also observed variations on DO cycles when fish were introduced to microcosms. He related the phenomenon to selective predation of autotrophic and heterotrophic organisms by fish. Increased water turbidity caused by fish disturbing microcosm soil substrate probably affected the DO cycle during this period. Tanks fertilized with rabbit excreta exhibited noticeably less suspended silt than did control tanks. Boyd (1979) reported that organic fertilizers act to clear water by adhering to soil particles in suspension.

#### Water pH

Daytime increases in pH values followed the same general pattern as those for DO (Table 1). No statistical differences were evident between treatments for early morning sample periods (7:00 AM). At the beginning of the experiment, the 50 and 75 kg treatments showed the highest afternoon pH increases, and the control the lowest ( $P < 0.05$ ). This pattern prevailed throughout the rest of the experiment in the 50 kg treatment, but not in the 75 kg treatment.

Table 1. Means and standard errors for physical parameters of water quality obtained from different rabbit excreta and control treatments. Each value is an average of bottom and top water layers for 3 replicates in each treatment.

| Parameter                        | Treatments              |                          |                         |                          |
|----------------------------------|-------------------------|--------------------------|-------------------------|--------------------------|
|                                  | Control                 | 50 kg.                   | 75 kg.                  | Pondclass                |
| Temperature at 07:00 (C)         | 24.9<br>( $\pm$ 0.04) a | 25.2<br>( $\pm$ 0.06) b  | 25.4<br>( $\pm$ 0.05) c | 25.1<br>( $\pm$ 0.04) ab |
| Temperature at 14:30 (C)         | 27.0<br>( $\pm$ 0.08) a | 27.4<br>( $\pm$ 0.09) ab | 27.6<br>( $\pm$ 0.09) b | 27.3<br>( $\pm$ 0.07) a  |
| pH at 07:00                      | 7.6<br>( $\pm$ 9.41) a  | 7.6<br>( $\pm$ 9.42) a   | 7.6<br>( $\pm$ 9.43) a  | 7.6<br>( $\pm$ 9.40) a   |
| pH at 14:30                      | 8.0<br>( $\pm$ 8.03) c  | 8.6<br>( $\pm$ 8.02) a   | 8.2<br>( $\pm$ 7.95) b  | 8.1<br>( $\pm$ 7.96) b   |
| Dissolved oxygen at 07:00 (mg/l) | 3.39<br>( $\pm$ 0.12) a | 2.97<br>( $\pm$ 0.11) ab | 2.56<br>( $\pm$ 0.10) b | 2.88<br>( $\pm$ 0.10) b  |
| Dissolved oxygen at 14:30 (mg/l) | 7.89<br>( $\pm$ 0.11) a | 8.01<br>( $\pm$ 0.10) a  | 6.9<br>( $\pm$ 0.16) b  | 7.71<br>( $\pm$ 0.10) a  |
| Dissolved oxygen at 20:30 (mg/l) | 7.05<br>( $\pm$ 0.11) a | 6.96<br>( $\pm$ 0.11) a  | 6.21<br>( $\pm$ .14) b  | 6.84<br>( $\pm$ 0.10) a  |

Values in each row with the same letter are not significantly different ( $P > 0.05$ )

## Alkalinity

Total alkalinity (mg/l  $\text{CaCO}_3$ ) in the water supply varied from 135 to 155 mg/l during the experimental period (Table 2, Figure 1). This variation was probably due to the high rainfall rate observed during the period. Total alkalinity in treatments receiving rabbit excreta was significantly different from the control treatment ( $P < 0.05$ ). Values as high as 275 mg/l were tabulated for microcosms fertilized at the 75 kg loading rate. On the other hand, much lower values were measured in the controls, 50 kg and Pondclass treatments.

## Dissolved Inorganic Nitrogen

Microcosms in the Pondclass treatment received a constant rabbit excreta loading rate averaging 26 kg/10,000  $\text{m}^3$ /day (dry matter), except in the first week when they received 37 kg/10,000  $\text{m}^3$ /day. The computed input rate followed the DO availability pattern in the different microcosms, and inorganic nitrogen and phosphorous generally exceeded the requirements for algal growth.

Mean total ammonia (mg/l  $\text{NH}_4\text{-N}$ ) concentrations in the 75 kg treatment were consistently much higher than other treatments (Table 2). No statistical differences were evident between the control, 50 kg and Pondclass treatments ( $P > 0.05$ ). The slight difference in ammonia concentration between Pondclass and 50 kg treatments could be attributed to nitrogen loss as  $\text{NH}_3$  by volatilization in the 50 kg treatment. Nitrogen losses by volatilization are accelerated by pH values greater than 8.5 (Boulding, et al. 1974; Schroeder, 1987) which were commonly tabulated for the 50 kg treatment. A tendency for ammonia to increase

through time is noted in the control, 50 kg, and Pondclass treatments, whereas it decreases from high initial values in the 75 kg treatment (Figure 2). Ammonia concentration in the control group was similar to that in 50 kg and Pondclass treatments. Nitrogenous metabolic wastes produced by fish and residual effects of uneaten food contributed to the ammonia nitrogen in the control treatment (Hepher, 1988).

### **Dissolved Inorganic Phosphorus**

Orthophosphate concentration was high in all treatments and higher in treatments receiving rabbit excreta than in the control ( $P < 0.05$ ). Clearly, orthophosphate levels increased in direct proportion with manuring rates (Figure 3).

### **Plant Production**

Estimates of mean gross and net primary productivity (reported as g C/m<sup>3</sup>/day) were statistically different ( $P < 0.05$ ) between treatments (Table 3). As a group, microcosms fertilized with 50 kg of rabbit excreta showed significantly higher productivities (3.89 and 1.67 for gross and net primary productivities, respectively) than control and 75 kg treatments, but not than Pondclass treatment ( $P > 0.05$ ). These differences in productivity between treatments generally existed throughout the experiment (Figures 4 and 5).

Terminal chlorophyll a concentrations at the 90 day interval were different between the control and fertilized treatments ( $P < 0.05$ ). The Pondclass and control treatments showed the highest and lowest values

Table 2. Total alkalinity, total ammonia, and phosphorous as orthophosphate for each treatment measured every 10 days. Each value is an overall average by treatment throughout the experimental period.

| Parameter                                     | Treatments         |                    |                    |                    |
|---|--------------------|--------------------|--------------------|--------------------|
|   | Control            | 50 kg.             | 75 kg              | Pondclass          |
| Total Alkalinity<br>(mg/l CaCO <sub>3</sub> ) | 193<br>(± 4.59) a  | 204<br>(± 4.72) ab | 219<br>(± 5.84) b  | 206<br>(± 4.85) ab |
| Total Ammonia<br>(mg/l NH <sub>4</sub> -N)    | 0.68<br>(± 0.05) a | 0.61<br>(± 0.05) a | 1.2<br>(± .39) b   | 0.66<br>(± 0.05) a |
| Orthophosphate<br>(mg/l)                      | 1.59<br>(± 0.07) a | 2.94<br>(± .16) b  | 4.58<br>(± 0.33) c | 2.99<br>(± 0.33) b |

Values in each row with the same letter are not significantly different ( $P > 0.05$ ).

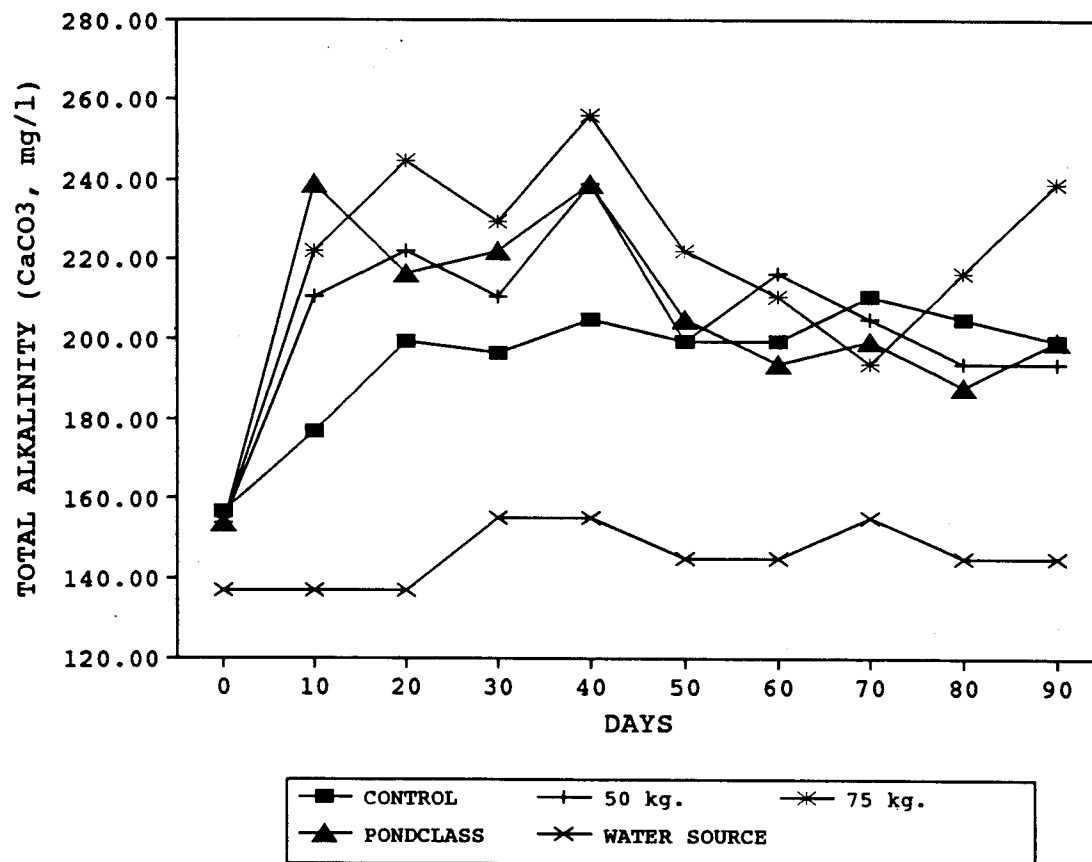


Figure 1. Trends in mean total alkalinity (mg/l, CaCO<sub>3</sub>) in the experimental microcosm and water source relationships measured every 10 days in a 90 day period. Each value is a mean of 3 replicates for each treatment.

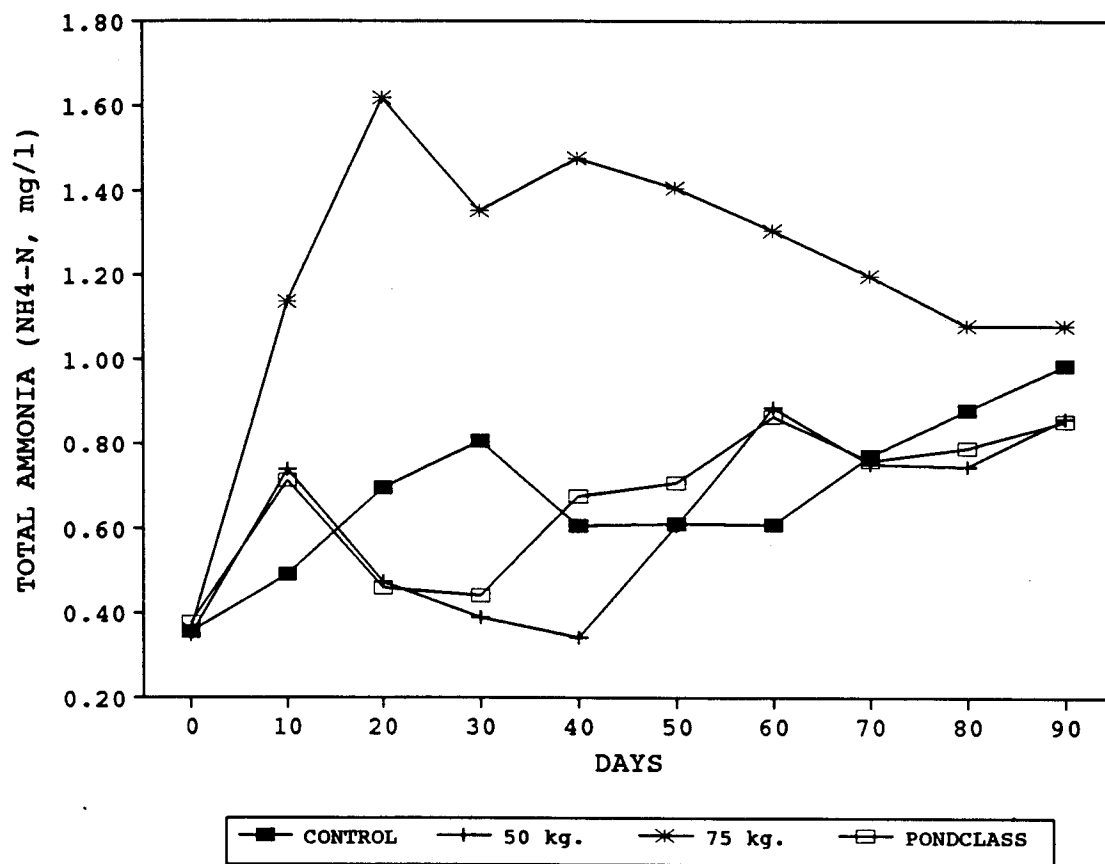


Figure 2. Trends in mean total ammonia (mg/l, NH<sub>4</sub>-N) in the experimental microcosms measured every 10 days in a 90 day period. Each value is a mean of 3 replicates for each treatment.

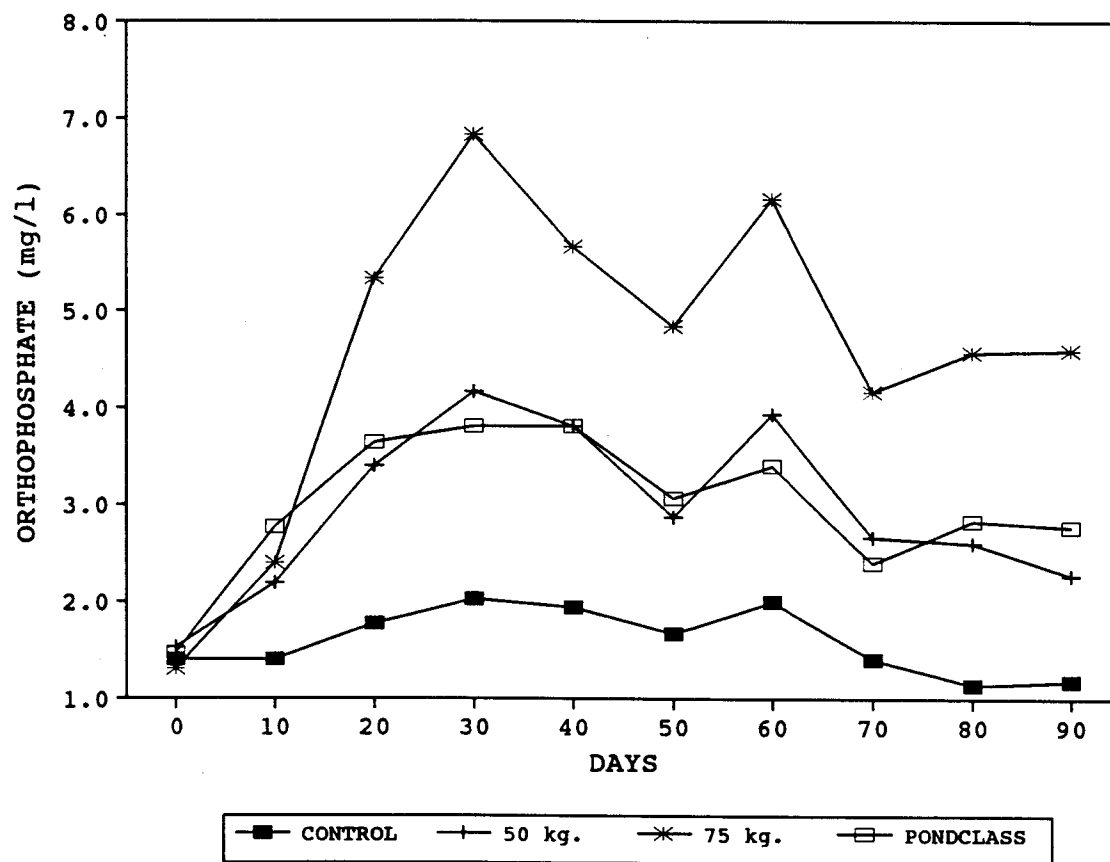


Figure 3. Mean orthophosphate in the experimental microcosms measured every 10 days in a 90 day period. Each value is a mean of 3 replicates for each treatment.

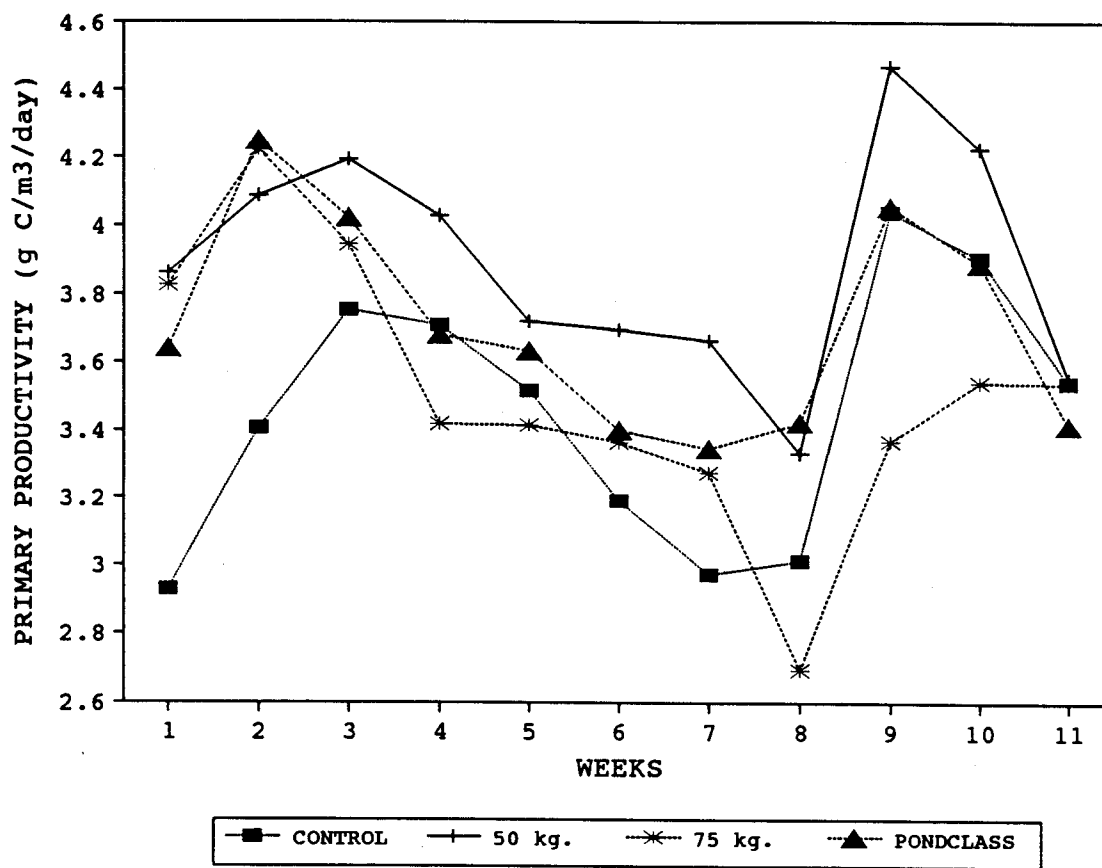


Figure 4. Mean gross primary productivity values for each treatment. Each values is a mean of 3 microcosms for treatment.

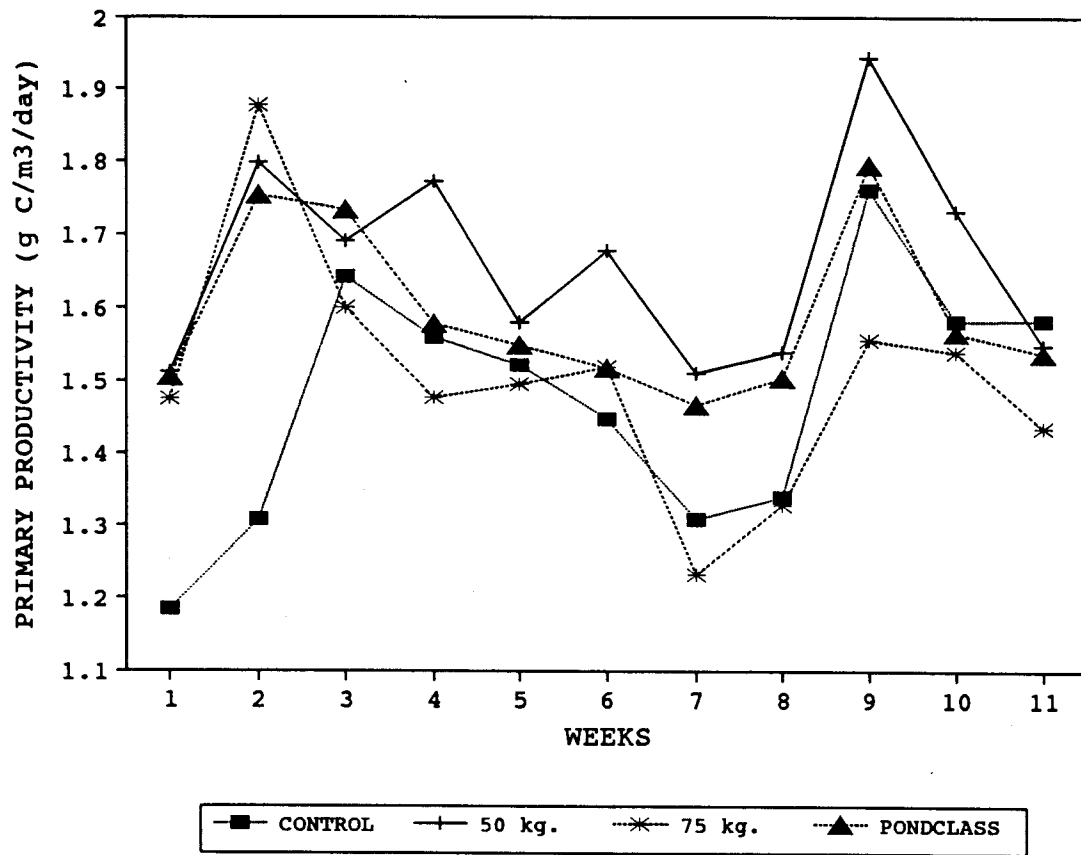


Figure 5. Mean net primary productivity values for each treatment. Each value is a mean of 3 microcosms per treatment.

chlorophyll a ( $242 \pm 38$  and  $116 \pm 11.2$  mg/m<sup>3</sup> respectively). There were not significant differences in chlorophyll a among fertilized treatments ( $P > 0.05$ ) (Table 3).

Occasionally, the development of euglenoid microorganisms was observed in microcosms. The occurrence of euglenoids during the experiment was independent of treatment type. Euglenoids prevailed throughout the experiment in one replicate of the Pondclass treatment and one tank of the control treatment.

Phytoplankton die-offs were observed in some tanks prior to fish stocking. These die-offs were likely related to thermal shock caused by topping off tanks with stream water that was generally 10 degrees C lower than water in the microcosms. To avoid this thermal shock a perforated PVC pipe connected to a hose and placed at an intermediate depth was used for subsequent water addition. This improved algal development during the remaining adaptation period.

### Fish Production

Net fish production in the control group was higher ( $0.0153$  kg/m<sup>2</sup>/day) than in 50 and 75 kg treatments, but similar to Pondclass ( $0.120$  kg/m<sup>2</sup>/day). No statistical differences in fish production were found between 50 and 75 kg treatments ( $0.1124$  and  $0.092$ , respectively) (Table 3).

Average percentage daily gain ( $\ln W_2 - \ln W_1 / \text{days}$ ) (APDG) was not different between treatments during the first 30-day interval. However, during the 60 and 90-day intervals fish in the 75 kg treatment grew more slowly than those in 50 kg and Pondclass treatments ( $P < 0.05$ )

Table 3. Summary of gross and net primary productivity, chlorophyll a concentration and fish production by treatment. Each value is a mean of 3 replicates ( $\pm$  SE).

| Variable   | Treatments                |                            |                           |                            |
|--|---------------------------|----------------------------|---------------------------|----------------------------|
|  | Control                   | 50 kg                      | 75 kg                     | Pondclass                  |
| Gross primary productivity<br>g C/m <sup>3</sup> /day      | 3.45<br>( $\pm$ 0.06) c   | 3.89<br>( $\pm$ 0.06) a    | 3.51<br>( $\pm$ 0.07) bc  | 3.70<br>( $\pm$ 0.06) ab   |
| Net primary productivity<br>g C/m <sup>3</sup> /day        | 1.48<br>( $\pm$ 0.03) b   | 1.67<br>( $\pm$ 0.03) a    | 1.50<br>( $\pm$ 0.03) b   | 1.59<br>( $\pm$ 0.03) ab   |
| Chlorophyll a <sup>1</sup><br>mg/m <sup>3</sup>            | 116<br>( $\pm$ 11.2) a    | 239<br>( $\pm$ 12.3) b     | 226<br>( $\pm$ 15.6) b    | 242<br>( $\pm$ 38.4) b     |
| Net fish production <sup>2</sup><br>kg/m <sup>2</sup> /day | 0.153<br>( $\pm$ 0.002) a | 0.1124<br>( $\pm$ 0.005) b | 0.092<br>( $\pm$ 0.004) b | 0.120<br>( $\pm$ 0.013) ab |
| Input conversion ratio <sup>3</sup><br>kg input/kg output  | 2.96<br>( $\pm$ 0.05) a   | 8.6<br>( $\pm$ 0.36) b     | 15.47<br>( $\pm$ 0.60) c  | 3.85<br>( $\pm$ .46) a     |
| Commercial production <sup>4</sup><br>kg/ha/day            | 17.0<br>( $\pm$ 0.27) a   | 12.5<br>( $\pm$ 0.54) b    | 10.1<br>( $\pm$ 0.40) b   | 13.34<br>( $\pm$ 1.47) ab  |

Values in each row with the same letter are not significantly different ( $P > 0.05$ ).

<sup>1</sup> Chlorophyll a (mg/m<sup>3</sup>) at 90-day interval.

<sup>2</sup> Values comprise total adult and progeny production.

<sup>3</sup> After Wohlfarth (1978); supplemental food and fresh excreta were considered similar

<sup>4</sup> Commercial production refers to extrapolated values of net production.

(Table 4). Growth rates in the Control, 50 kg and Pondclass treatments were not significantly different ( $P < 0.05$ ). A two-way analysis of variance detected that the slight differences in total fish weight gain between treatments in the first 30 days were strongly influenced by gender ( $P = 0.0074$ ), but not by treatment ( $P > 0.05$ ). However, the same statistical analysis applied to the 60 and 90-day intervals, indicated that fish growth performances were related to both treatment and sex effects ( $P < 0.001$ ), but with no 2-factor interaction ( $P > 0.05$ ). The APDG for female fish corresponded to 75 , 55, 38, and 64 % of the measured APDG for males in control, 50 kg, 75 kg, and Pondclass treatments, respectively. Figure 6 depicts the mean monthly body weight changes by treatment, including both sexes, during the experimental period. Female weight changes by treatment are shown in Figure 7.

Fish condition indices by treatment and sex were not uniform and did not follow the trends of fish growth performance (Table 5, Figure 8). Overall condition index estimates declined in the first 30 days, probably as a consequence of low initial system productivity and high competition for food at this high stocking rate. Condition factor estimates for males was not different between treatments during the 90-day experimental period ( $P > 0.05$ ). Estimates for females fluctuated widely over time (Figure 9) and, interestingly, were inversely related to progeny production (Table 6). At the end of the experimental period, statistical differences in condition factor were seen for females between control and fertilized treatments ( $P < 0.05$ ).

Table 4. Growth response of O. niloticus for the each assessed treatment.  
Values given as means ( $\pm$  SE).

| Variable                   | Treatments                  |                              |                             |                              |
|----------------------------|-----------------------------|------------------------------|-----------------------------|------------------------------|
|                            | Control                     | 50 kg                        | 75 kg                       | Pondclass                    |
| Weight, initial (g)        | 34.9<br>( $\pm$ 0.54)       | 34.9<br>( $\pm$ 0.54)        | 34.9<br>( $\pm$ 0.54)       | 34.9<br>( $\pm$ 0.54)        |
| Weight, 30 days (g)        | 50.79<br>( $\pm$ 1.63) a    | 46.34<br>( $\pm$ 1.63) a     | 45.05<br>( $\pm$ 1.84) a    | 48.83<br>( $\pm$ 1.71) a     |
| Weight, 60 days (g)        | 59.7<br>( $\pm$ 1.68) a     | 53.3<br>( $\pm$ 1.95) ab     | 48.5<br>( $\pm$ 2.15) b     | 55.01<br>( $\pm$ 2.07) ab    |
| Weight, 90 days (g)        | 63.0<br>( $\pm$ 1.74) a     | 56.3<br>( $\pm$ 2.01) ab     | 51.4<br>( $\pm$ 2.01) b     | 58.1<br>( $\pm$ 1.85) ab     |
| APDG, 30 days <sup>1</sup> | 0.012<br>( $\pm$ 0.001) a   | 0.009<br>( $\pm$ 0.001) ab   | 0.008<br>( $\pm$ 0.001) b   | 0.011<br>( $\pm$ 0.001) ab   |
| APDG, 60 days              | 0.0089<br>( $\pm$ 0.0005) a | 0.0069<br>( $\pm$ 0.0006) ab | 0.005<br>( $\pm$ 0.0008) b  | 0.007<br>( $\pm$ 0.0006) ab  |
| APDG, 90 days              | 0.0065<br>( $\pm$ 0.0003) a | 0.0052<br>( $\pm$ 0.0004) ab | 0.0042<br>( $\pm$ 0.0004) b | 0.0056<br>( $\pm$ 0.0003) ab |

<sup>1</sup> APDG= Average percentage daily gain (Winberg, 1971)

Values in each row with the same letter are not significantly different ( $P > 0.05$ )

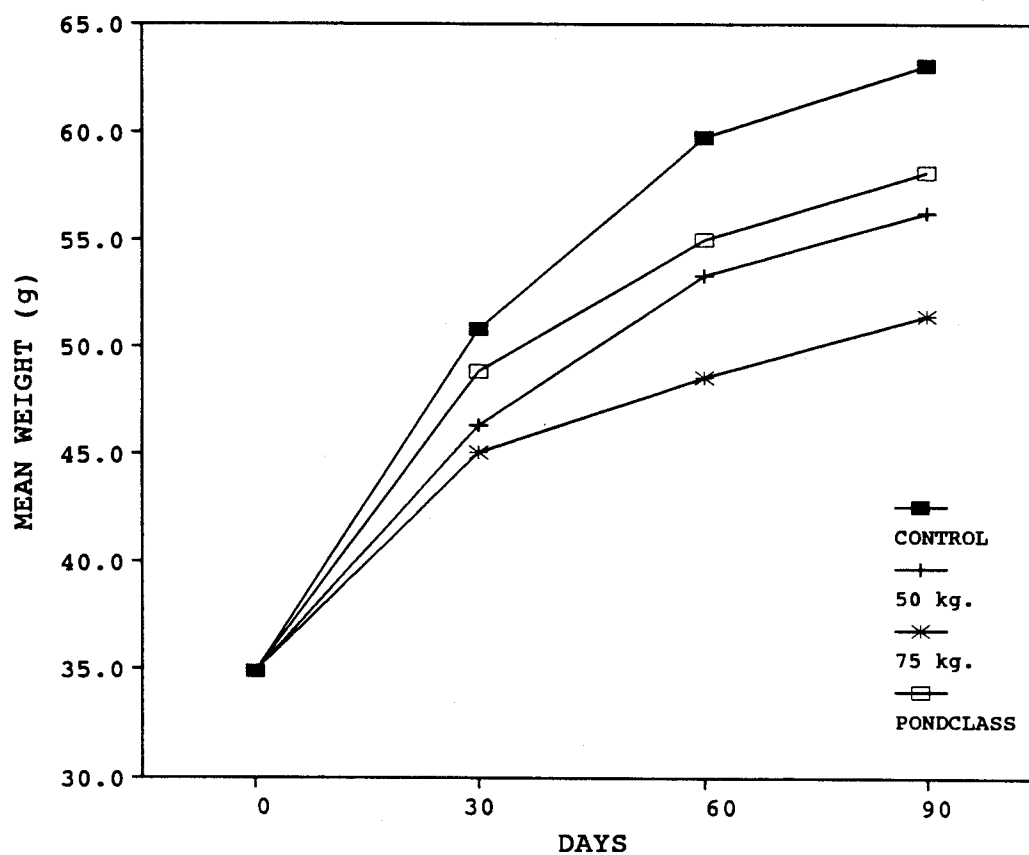


Figure 6. Mean monthly body weight of Nile tilapia (*O. niloticus*) both sexes included.

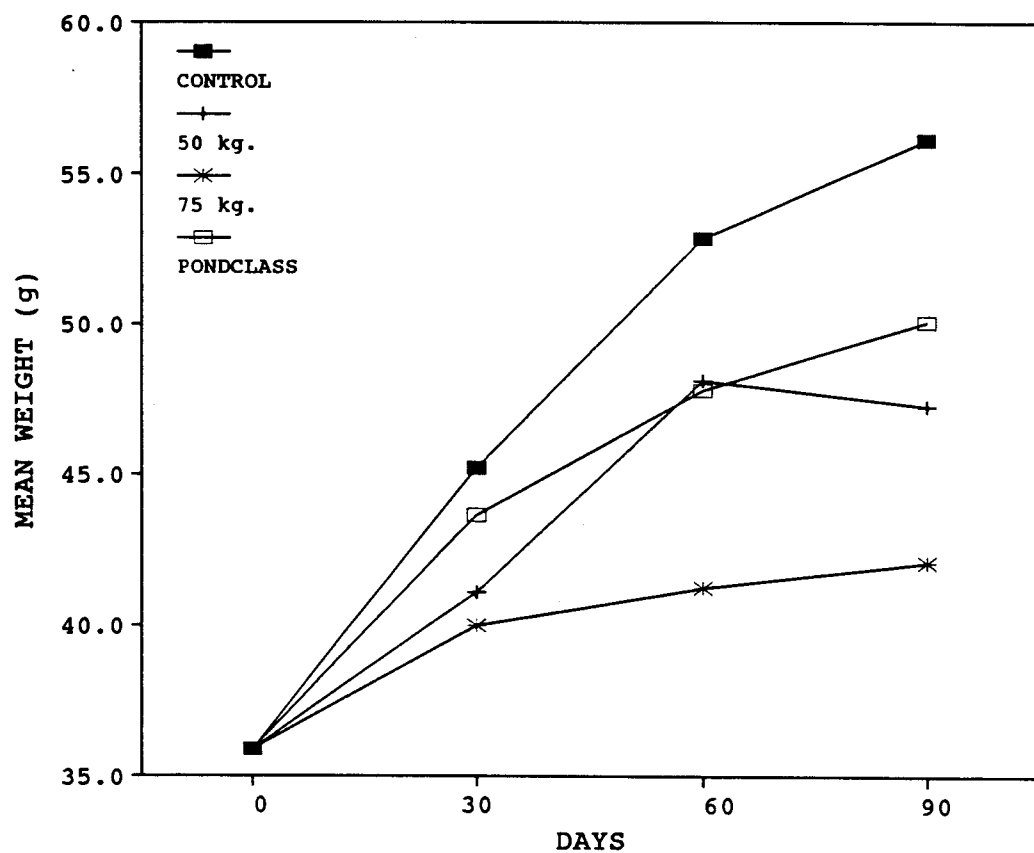


Figure 7. Mean monthly body weight of Nile tilapia (*O. niloticus*) (females) for each treatment.

Table 5. Condition indices (K) per sex for O. niloticus for each sampling period by treatment. Values given as means ( $\pm$  SE)

| Period  | Sex     | Treatments          |                     |                     |                     |
|---------|---------|---------------------|---------------------|---------------------|---------------------|
|         |         | Control             | 50 kg.              | 75 kg.              | Pondclass           |
| Initial | males   | 3.5 ( $\pm$ 0.05) a | 3.5 ( $\pm$ 0.05) a | 3.5 ( $\pm$ 0.05) a | 3.5 ( $\pm$ 0.05) a |
| Initial | females | 3.4 ( $\pm$ 0.04) a | 3.4 ( $\pm$ 0.04) a | 3.4 ( $\pm$ 0.04) a | 3.4 ( $\pm$ 0.04) a |
| 30 days | males   | 3.4 ( $\pm$ 0.07) a | 3.2 ( $\pm$ 0.09) a | 3.2 ( $\pm$ 0.09) a | 3.2 ( $\pm$ 0.10) a |
| 30 days | females | 3.0 ( $\pm$ 0.10) a | 3.1 ( $\pm$ 3.24) a | 2.9 ( $\pm$ 0.10) a | 3.2 ( $\pm$ 0.10) a |
| 60 days | males   | 3.4 ( $\pm$ 0.07) a | 3.3 ( $\pm$ 0.07) a | 3.2 ( $\pm$ 0.08) a | 3.3 ( $\pm$ 0.04) a |
| 60 days | females | 3.4 ( $\pm$ 0.13) a | 3.4 ( $\pm$ 0.09) a | 3.0 ( $\pm$ 0.11) a | 3.3 ( $\pm$ 0.07) a |
| 90 days | males   | 3.3 ( $\pm$ 0.05) a | 3.2 ( $\pm$ 0.03) a | 3.1 ( $\pm$ 0.05) a | 3.2 ( $\pm$ 0.07) a |
| 90 days | females | 3.4 ( $\pm$ 0.08) a | 2.9 ( $\pm$ 0.05) b | 2.8 ( $\pm$ 0.12) b | 3.0 ( $\pm$ 0.07) b |

Values in each row with same letter are not significantly different ( $P > 0.05$ ).

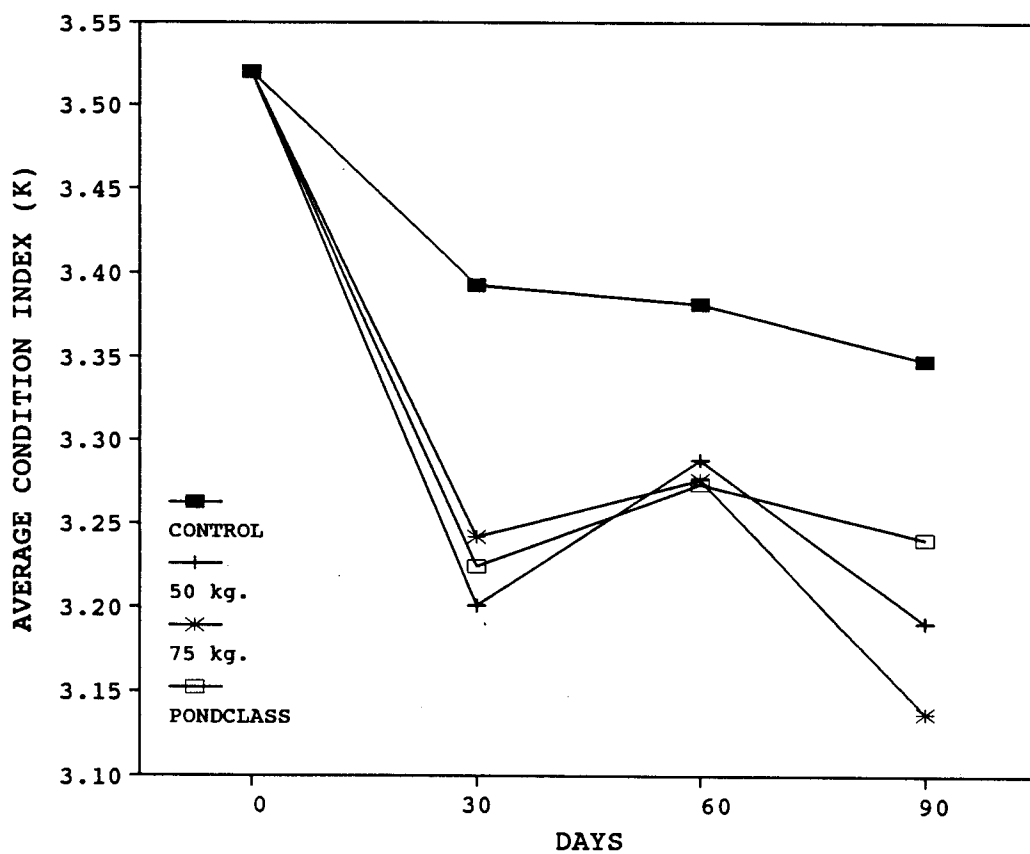


Figure 8. Mean monthly condition index of Nile tilapia (*O. niloticus*) (males) for each treatment.

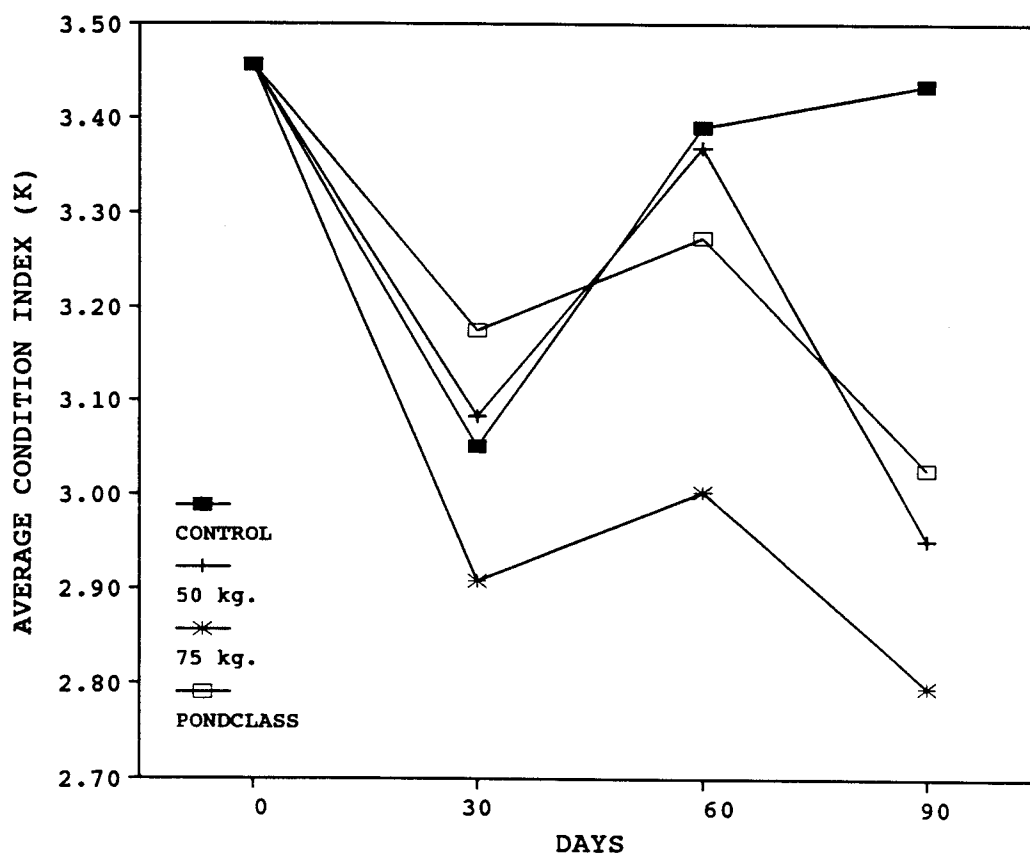


Figure 9. Mean monthly condition index of Nile tilapia (*O. niloticus*) (females) for each treatment.

Table 6. Mean number progeny ( $\pm$  SE) of *O. niloticus* collected from 6 harvests and overall progeny production per female during 90-day experimental period. Each mean is an average of 3 replicates.

| Harvest                   | Treatments             |                        |                        |                       |
|---------------------------|------------------------|------------------------|------------------------|-----------------------|
|                           | Control                | 50 kg.                 | 75 kg.                 | Pondclass             |
| 1                         | 0                      | 0                      | 107<br>( $\pm$ 45)     | 0                     |
| 2                         | 214<br>( $\pm$ 113)    | 116<br>( $\pm$ 22)     | 159<br>( $\pm$ 52)     | 161<br>( $\pm$ 32)    |
| 3                         | 0                      | 82<br>( $\pm$ 16)      | 64<br>( $\pm$ 39)      | 94<br>( $\pm$ 26)     |
| 4                         | 108<br>( $\pm$ 42)     | 89<br>( $\pm$ 49)      | 212<br>( $\pm$ 52)     | 94<br>( $\pm$ 26)     |
| 5                         | 210<br>( $\pm$ 45)     | 122<br>( $\pm$ 63)     | 158<br>( $\pm$ 82)     | 0                     |
| 6                         | 251<br>( $\pm$ 68)     | 141<br>( $\pm$ 62)     | 309<br>( $\pm$ 82)     | 132<br>( $\pm$ 38)    |
| Total progeny<br>(number) | 783<br>( $\pm$ 115) ab | 550<br>( $\pm$ 169) ab | 1009<br>( $\pm$ 84) b  | 387<br>( $\pm$ 41) a  |
| Progeny/female/<br>day    | 4.3<br>( $\pm$ 0.64)ab | 3.0<br>( $\pm$ 0.93)ab | 5.6<br>( $\pm$ 0.47)ab | 2.1<br>( $\pm$ 0.23)a |

Values in each row with the same letter are not significantly different ( $P > 0.05$ ).

Reproduction occurred in all microcosms. Total progeny production and progeny/female/day were highest in microcosms fertilized at the 75 kg loading rate (Table 6). Time intervals between broods produced in this treatment averaged 15 days. Fewer young were collected from the Pondclass treatment ( $387 \pm 41$ ). There was no apparent relationship between progeny number and the net fish production by adults.

### Characteristics of Rabbit Excreta

A consistent difference in manure conversion ratio was detected between treatments fertilized with rabbit excreta ( $P < 0.05$ ). The Pondclass and 75 kg treatments showed the lowest and highest ratios, (3.85 and 15.47, respectively). Further, on a wet weight basis, Pondclass was not statistically different from the control group ( $P > 0.05$ ). There was no clear relationship between net primary productivity and fish production. However, a weak negative relationship between nitrogen input and net fish production was detected ( $R^2 = 0.48$ ).

The dry matter content in rabbit excreta used to fertilize the microcosms averaged 22.5 % ( $\pm 0.23$ ) (Table 7). As ambient air temperature in the rabbit rearing facility went above 28 C, the moisture content in rabbit excreta increased. The rabbit excreta dry matter content, excluding the urine plus water waste fraction, averaged 52.9 % ( $\pm 0.010$ ). However, inclusion of the water waste fraction reduced the dry matter content to 28.4 % ( $\pm 0.006$ ). The mean ratio of urine plus water waste to fecal matter was 1:1 on a wet weight basis (Table 8).

Nitrogen content in rabbit excreta varied according to rabbit size, and the presence or absence of urine plus water waste, and food

droppings. Nitrogen content in feces from rabbits in grower-finisher and adult categories was greater (3.23 and 2.81 %, respectively) than rabbits weighing less than 1 kg per animal (2.35 and 2.11 % for a doe plus its litter, and individual rabbits weighing less than 1 kg, respectively). Nitrogen in urine averaged 28 % (SD 13) of the total nitrogen in excreta (Table 8). Similarly, nitrogen provided by food droppings contributed 12 % (SD 0.05) of the total nitrogen in excreta.

A mean of 1.96 (SD 0.07) was determined for total phosphorous concentration in complete (feces, urine plus water, and food droppings) rabbit excreta samples (Table 7).

The manure output (dry matter) as % total live weight (TLW) was inversely related to rabbit size. In the limited number of samples taken, this manure output/TLW percentage was higher in small size animals (10.6 and 6 % for a doe plus its litter, and rabbits weighing less than 1 kg per animal, respectively), than in grower-finisher, and adult animals (3.8 and 2.69 % respectively).

Table 7. Dry matter, total phosphorous, and total nitrogen contents for complete <sup>1</sup> rabbit excreta samples for each experimental phase.

| Parameter           | First Phase         | Second Phase        |
|---------------------|---------------------|---------------------|
| Dry matter %        | 22.47 ( $\pm$ 0.23) | 28.4 ( $\pm$ 0.006) |
| Total phosphorous % | 1.96 ( $\pm$ 0.07)  | nd                  |
| Total nitrogen %    | 2.11 ( $\pm$ 0.03)  | 2.45 ( $\pm$ 0.06)  |

<sup>1</sup> Complete rabbit excreta samples included fecal matter, urine plus water waste, and food droppings.

nd = not determined

Table 8. Summary of selected rabbit excreta manure characteristics based on rabbit total live weight (TLW), presence or absence of urine plus water waste, and food droppings evaluated during the second experimental phase. Values expressed as means ( $\pm$  SE) or (SD  $\pm$  = standard deviation)

| Parameter  | Rabbit                    |                           |                                   |                          |
|--|---------------------------|---------------------------|-----------------------------------|--------------------------|
|  | Doe + litter <sup>1</sup> | Weanlings<br>(TLW < 1 kg) | Grower-finisher<br>(TLW < 2.3 kg) | Adults<br>(TLW > 2.5 kg) |
| Dry matter % complete sample                         | 32.7 ( $\pm$ 0.07)        | 33.8 ( $\pm$ 0.08)        | 24.9 ( $\pm$ 0.01)                | 24.7 ( $\pm$ 0.02)       |
| Dry matter % no urine + water waste                  | 53.1 ( $\pm$ 0.05)        | 56.2 ( $\pm$ 0.02)        | 50.9 ( $\pm$ 0.02)                | 50.9 ( $\pm$ 0.012)      |
| Total nitrogen % <sup>2</sup> complete sample        | 2.11                      | 2.35                      | 3.23                              | 2.81                     |
| Total nitrogen % no <sup>2</sup> urine + water waste | 1.85                      | 1.71                      | 1.79                              | 1.99                     |
| Total nitrogen % no <sup>2</sup> food droppings      | 1.92                      | 2.08                      | 2.60                              | 2.52                     |
| Total nitrogen % urine <sup>3</sup>                  | 0.14<br>(SD 0.01)         | 0.39<br>(SD $\pm$ 0.04)   | 0.55<br>(SD $\pm$ 0.06)           | 0.65<br>(SD $\pm$ 0.04)  |
| Excreta output/TLW % <sup>4</sup>                    | 10.5<br>( $\pm$ 0.8)      | 6.00<br>( $\pm$ 0.13)     | 3.98<br>( $\pm$ 0.18)             | 2.69<br>( $\pm$ 0.02)    |
| Urine/feces ratio (fresh matter)                     | 0.78<br>( $\pm$ 0.02)     | 1.16<br>( $\pm$ 0.06)     | 0.95<br>( $\pm$ 0.011)            | 1.16<br>( $\pm$ 0.084)   |

<sup>1</sup> Doe and its litter averaged 7.25 kg TLW (final weight - initial weight/2).

<sup>2</sup> Only a pooled sample was analyzed.

<sup>3</sup> Nitrogen content as Kjeldahl nitrogen.

<sup>4</sup> Rabbit excreta expressed as dry matter.

## DISCUSSION

In this section the results of this investigation will be discussed in the context of the stated objectives. The objectives were: (1) to evaluate the use of microcosms as a tool for investigation the dynamics of fertilized tropical ponds, and (2) to determine the suitability and characteristics of rabbit excreta for use as an organic fertilizer for culture of Nile Tilapia.

### Microcosm Evaluation

The data presented in the previous section indicate that the primary production and fish production characteristics of the microcosms investigated in this project were very similar to real ponds which received similar manure loadings. In comparing the performance of the microcosms to reported observations with real ponds, it must be recognized that the rabbit excreta loading rates and fish stocking rates used here were relatively high compared to most aquaculture situations. The deviation from traditional practice was intentional. The fish stocking density was determined by the statistical design of the experiments. The 50 kg and 75 kg loading rates were selected to bracket the maximum allowable loading for rabbit excreta, and were based on preliminary experiments of shorter duration which indicated that the maximum loading was likely to be greater than 50 but less than 75 kg/10,000 m<sup>3</sup>/day. The maximum loading rate does indeed appear to be intermediate between these two treatments. Both primary productivity

(Table 3) and fish growth rates (Table 4) were significantly lower in the 75 kg treatment than in the 50 kg treatment. Additionally, early morning dissolved oxygen (Table 1), and ammonia nitrogen (Table 2) concentrations approached or reached critical levels in the 75 kg treatment, characteristic of over fertilization.

Results from microcosms experiments should be properly scaled for comparison with data from typical earthen ponds. Comparison of the primary productivities shown in Table 3, which are reported in volumetric dimensions, with literature reported values is facilitated by converting the former to surface area dimensions. This is necessary because the 1.4 m<sup>2</sup> microcosms were only 0.48 m deep, whereas aquaculture ponds are typically about 1 meter deep. Thus direct comparison on a volume basis may introduce errors of up to twofold. The net productivities of the control, 50 kg, 75 kg and Pondclass treatments were 0.69, 0.75, 0.70, and 0.74 g C/m<sup>2</sup>/day, respectively. These values are slightly lower than those reported for typical tropical pond systems such as the 0.93 and 1.3 g C/m<sup>2</sup>/day reported by McNabb et al. (1988) and by Teichert-Coddington et al. (1991) for Indonesia and Panama, respectively. They are substantially lower than the 2.89 g C/m<sup>2</sup>/day reported by Hepher (1962) for intensively-manured ponds in Israel.

The primary productivity in the control treatments is associated with primary nutrients leaching from the trout-prepared food and from ammonia excreted by the fish (Figures 4 and 5). Un-ingested food appears to have served as a fertilizer, providing nutrients for algal growth. Previous workers have demonstrated that fish do not feed well immediately after their introduction into new environments (Ishitawa,

quoted by Hepher, 1988). Additionally, Schroeder (1979; and 1983) emphasized the preference for phytoplankton and zooplankton, shown by fish, even when supplemental food is present. Phytoplankton and zooplankton development in the microcosms during the adaptation period may help to explain the low prepared-food consumption by fish in the control treatment, and the similar fish growth between treatments during the first 30 days.

Microcosms fertilized with rabbit excreta showed different patterns of primary productivity than the control treatment. The relatively low values of gross and net primary productivity relative to values reported for real ponds indicates that some factor was limiting productivity in the microcosms.

Primary nutrients do not appear to have limited primary productivity in the fertilized treatments. Total alkalinity (methyl orange alkalinity) exceeded the values reported as limiting primary productivity (Boyd, 1979). Therefore, inorganic carbon must not have been a limiting nutrient in this study. Similarly, nitrogen and phosphorus were present in excess in the different treatments, but were not completely utilized by algae. The rabbit excreta N:P ratio of 1:1 (Table 7) indicates that the algal requirements for phosphorus were fulfilled.

Light energy appears to be the factor limiting primary production in the microcosms, especially in the 50 and 75 kg treatments. The average values on net primary productivity and chlorophyll *a* concentration among fertilized treatments (Table 3) were very similar in spite of having receiving different rabbit excreta loading rates. This

indicates algae were not able to transform the inorganic nutrients into new organic matter at a rate sufficient to reduce water nutrient concentrations. Therefore, inorganic carbon, nitrogen (as ammonia), and phosphorus were present in adequate amounts to result in greater algal growth if light levels had not limited the photosynthetic process (Tables 2 and 3, Figures 4 and 5).

The surprising general increase in primary productivity observed on weeks 8 and 9 coincided with the change in food and rabbit excreta application routine during week 8, suggesting that fertilization frequency may influence phytoplankton growth. Further studies are needed to test this hypotheses.

The fish growth performance observed in the different treatments in this study resembles observations from real earthen ponds. The average percentage daily gain (APDG) was influenced by fish physiological and intrinsic effects of the treatments. As mentioned previously, APDG and net fish production (Table 4 and 3 respectively) excepting the first 30 days, were influenced by both gender and treatments. A distinct difference in growth rate between sexes occurs in *Tilapia* species; females grow slower than males (Hepher, 1988). The individual growth rates of the females in the different treatments clearly influenced the net fish yield in the microcosms. Additionally, given the high fish stocking density utilized, APDG tended to be low, but net fish yield was compensated by greater biomass.

Treatment effects on fish APDG and net fish production may be divided according to the sources of the effects. First, there were not statistical differences in fish weight gain among the control, 50 kg,

and Pondclass treatments. The values of primary productivity and chlorophyll a (Table 3) suggest that fish weight gain in the control treatment was determined in part by the abundant natural food available in the microcosms, reaffirming the importance of measuring the natural food potential in order to minimize the supplemental food usage in O. niloticus culture (Moav, et al. 1977; Schroeder, 1979 and 1983; Hepher, 1988).

Second, the differences in APDG and net fish yield among fertilized treatments are likely related to water quality deterioration. Dissolved oxygen at dawn ( $< 2$  ppm) may have severely affected fish condition in microcosms fertilized at the 75 kg loading rate. Although O. niloticus have the ability to survive at very low DO concentrations, they usually become lethargic and do not feed well until DO levels are recharged through photosynthesis (Ross and Ross, 1983; Hepher, 1988). Animal nitrogenous metabolic products such as non-ionized ammonia at levels of greater than 0.06 mg/l may depress growth in Ictalurus punctatus (Colt and Tchobanoglous, 1978) and also in O. niloticus (Abdalla, quoted by Batterson, et al 1989). Using Trussell's (1972) information (at 25 C) experimental data (Table 1) for pH at 14:30, and total ammonia data (Table 2), calculated values of non-ionized ammonia for the treatments were 0.03, 0.11, 0.10, and 0.04 mg/l for the control, 50 kg, 75 kg, and Pondclass treatments, respectively. These values indicate fish in microcosms receiving the 50 and 75 kg loading rates were exposed to non-ionized ammonia levels high enough to explain the poor weight gain observed. These values also suggest that although primary productivity and chlorophyll a were similar in the 50 kg and

Pondclass treatments (Table 3) ammonia toxicity may have contributed to the weight gain difference between treatments. The decline observed in female condition indices and weight gain after the 60 day interval in microcosms receiving the 50 kg loading rate may also be associated with ammonia toxicity (Figures 7 and 9)

Reproduction similarly impoverished the adult fish net production in microcosms fertilized with the rabbit excreta. In addition to egg production, parental behavior and grazing in females results in energy expenditures devoted to reproduction in fish species such as *Tilapia* (Wootton, 1985). Evidently, females in treatments fertilized at 50 and 75 kg loading rates diverted a large fraction of energy from somatic tissue into gonadal tissue (Tables 4 and 5, and Figure 9). It is noteworthy that the fluctuations in condition indices of females coincided with the timing of offspring production (Table 6 and Figure 9). The increasing condition indices of females in the control treatment supports other reported observations that supplying supplemental food helps to maintain growth rate and offspring productions (Wootton, 1985). Condition indices of males fish in microcosms fertilized according to the Pondclass program tended to follow the same pattern as that for fish fed on trout-pellet food in the control treatment. On the other hand, male fish condition indices in treatments fertilized at 50 and 75 kg loading rates declined after the 60 day interval, probably because of water quality deterioration.

The APDGs observed in this study are comparable to reported field experiences. For example, Stickney, et al. (1979) and Hesby and Stickney (1980) reported APDG values for *Tilapia* ranging from 0.0042 to 0.0049 in

earthen ponds fertilized with poultry waste or pig manure. The fish stocking densities for their experiments were 8,000 fish/ha. Hanson et al. (1989) reported a fish net yield of 2.04 kg/ha/day for O. niloticus in Rwandan earthen ponds. By contrast, Delgado (1985) and Msiska and Cantrell (1985) reported fish yield between 5.6 and 5.6 and 7.3 kg/ha/day, respectively, in Tilapia culture in concrete tanks. These reported values are lower than the 10.1 and 13.34 kg/ha/day obtained in this study for the 75 kg and Pondclass treatments, respectively, (Table 3). The fish yields observed in this study are similar to the 15 to 20 kg/ha/day obtained in Tilapia culture in Philippines utilizing poultry and pig manures (Hopkins and Cruz, 1982). However, the results are lower than fish yields reported for intensively-manured and chemically fertilized ponds. For example, Moav, et al. (1977) and Barash, et al. (1982) reported fish yields of 32 kg/ha/day in a systems utilizing cow manure plus chemical fertilization, and 38 kg/ha/day in an integrated duck and fish farming system. Both experiments were carried out in polyculture systems. Assuming constant productivity and fish production throughout the year, the extrapolated fish yields observed in this study would correspond to 6,205, 4,563, 3686, and 4,869 kg/ha/year for control, 50 kg, 75 kg, and Pondclass treatments, respectively.

The food conversion ratio observed in the control treatment 2.96 coincides with conversion ratios reported in the aquaculture literature. For example, Moav, et al. (1977) reported estimates ranging from 1.8 to 2.9 for high-protein pellets in polyculture systems. The Pondclass treatment showed the lowest manure conversion ratio. This conversion ratio 3.85 (Table 3) would be considered highly satisfactory for animal

and fish integrated systems (Wohlfarth, 1978; Woynarovich, 1979).

Inefficient use of rabbit excreta and poor weight gain occurred in the microcosms fertilized at 50 and 75 kg rabbit excreta loading rates resulting in large conversion ratios.

The reproduction rates in the fertilized treatments increased proportionally to the rabbit excreta loading rate (Table 6). Stickney and Hesby (1977) and Dettweiler and Diana (1991) observed the same reproduction tendency in Nile tilapia raised in chicken and swine-manured earthen ponds. The high reproduction rate observed in the 75 kg treatment may be related to either input loading rate (as mentioned above) or external factors such as water temperature. Temperatures fluctuations of 3 to 7 degrees C in water temperatures at dawn and at noon respectively, and commonly recorded for the 50 and 75 kg treatments may have led to maximum offspring production (Hughes, et al. 1983)

#### Characterization of Rabbit Excreta

The fish in this study were observed feeding selectively on rabbit excreta. That is, fish did not eat fecal pellets voided by adult rabbits but did consume fecal pellets voided by young animals (kits and weanling rabbits). This suggests that O. niloticus responded to different feeding attractants such as the milk residuals and derivatives present in fecal pellets voided by young fish. But further studies are necessary to examine this hypothesis.

An advantageous feature of rabbit excreta for aquacultural purposes seems to be the low specific gravity of the pellets. Rabbit fecal pellets are buoyant wastes. This property favors the leaching or

more inorganic nutrients into water relative to non-buoyant wastes that become buried in sediments. The buoyant wastes probably enrich the nutrient pool available for algal growth more quickly and to a greater degree than non-buoyant types. Additionally, rabbit excreta may contain high bacterial protein bio-converted in the cecum and colon that might hasten the excreta degradation process in earthen ponds (Swick, et al., 1978; Straw, 1988).

Fluctuations in dry matter percent and nitrogen content of rabbit excreta were probably in response to environmental conditions (Table 7). Rabbits are sensitive to temperature changes. Temperatures above 28 C reduce food consumption, but increases water consumption (Lebas, 1983; Gilbertson and Clemens, 1986).

Dry matter also varied according to the presence or absence of urine plus water waste. Dry matter content of 52.9 % ( $\pm 0.01$ ) without urine plus the water fraction is very close to that value reported by Lannan, et al. (1991). However, the dry matter content in samples which included urine plus water waste was only 28.4 % ( $\pm 0.006$ ). The urine:fecal matter ratio should be considered when rabbit excreta is used to fertilize aquatic systems because urine may determine the efficiency of the use of fecal matter by autotrophic organisms.

Clearly the addition of urine plus water waste increased the rabbit excreta nitrogen content (Table 8). Nitrogenous compounds in rabbit urine include ammonia, urea, and amino acids (Chawan, 1979; Straw, 1988). Therefore, urine is the most important component of rabbit excreta for fertilizing aquacultural systems, because it provides inorganic nitrogen sources readily utilized by algae.

Additionally, urine contains about 70 percent of the salt content of rabbit wastes (Gilbertson and Clemens, 1986), wherein calcium salts and derivatives constitute a large fraction (Cheeke, 1987).

Food droppings provided 12 percent (Table 8) of the total nitrogen in complete rabbit excreta samples. However, unlike urine food droppings contain more organic nitrogen (as protein) which may be directly utilized by organisms such as fish. Suckling and weanling rabbits probably wasted more food, but the amount could not be measured in this experiment.

The phosphorous content of  $1.96 (\pm 0.007)$  mg/l and N:P ratio of approximately 1:1 (Table 7) indicate that rabbit excreta can supply adequate phosphorous for algal growth when no other factors limit its bio-availability in water.

The rabbit excreta output/TLW (Table 8) relationship is a helpful tool to determine the number of animals to keep per water surface area in integrated systems, and to adjust the number of animals over the fish ponds to minimize risks to fish health. However, the results of this study should be considered conservatively because the animal sample size and short experimental period limited the statistical power of the experiment.

In summary, the nitrogen content of complete rabbit excreta (fecal matter, urine plus water waste, and food droppings) resembles that of poultry manure and poultry litter as reported by ASAE (1990). However, its phosphorous content is higher than that of other animal manures utilized as organic fertilizer in fish ponds.

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## **APPENDIX**

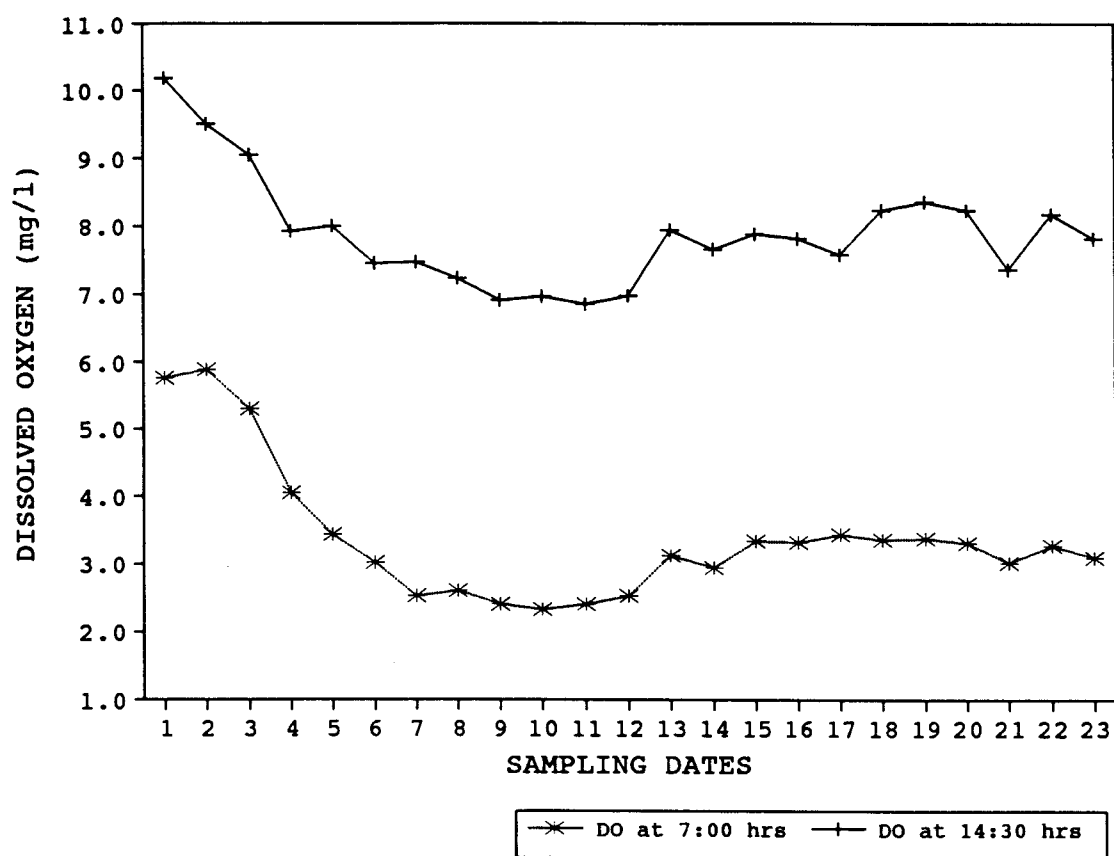


Figure 10. Weekly mean dissolved oxygen concentrations at 07:00 and 14:30 hrs for the control treatment. Each value is an average of bottom and surface water layers.

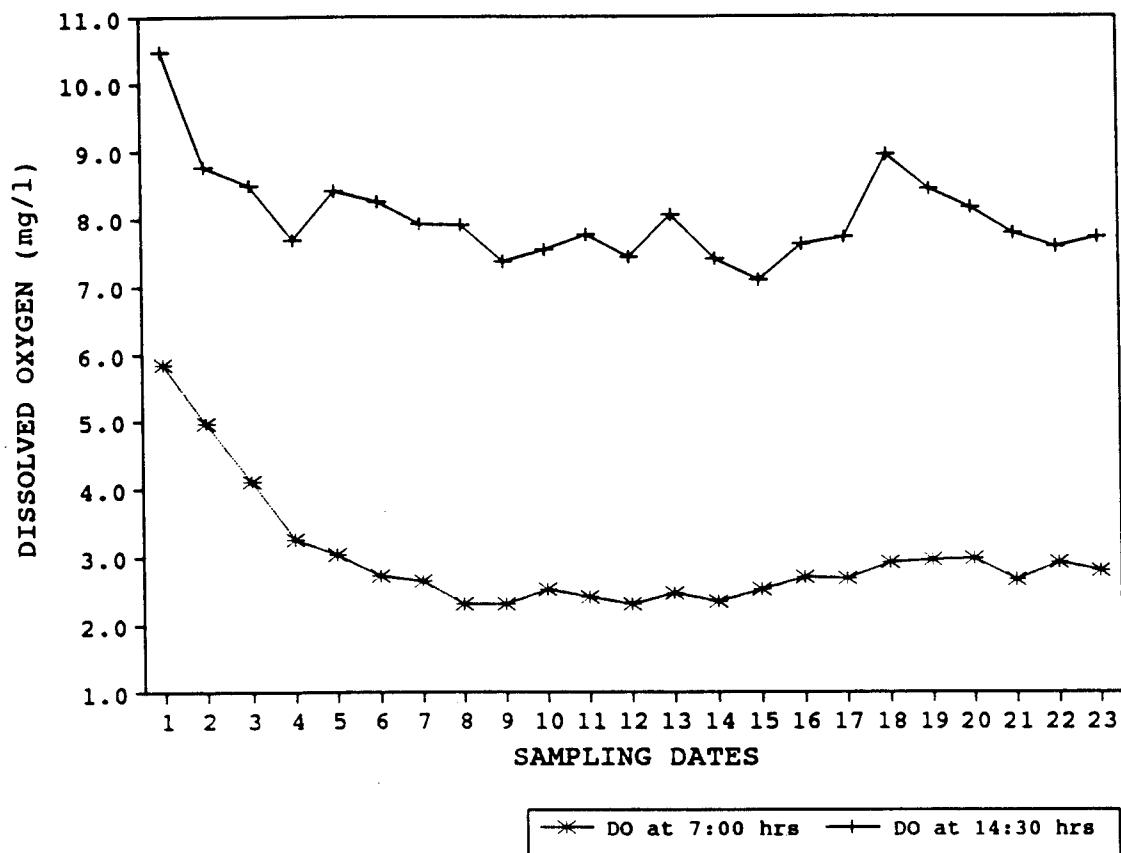


Figure 11. Weekly mean dissolved oxygen concentration at 07:00 and 14:30 hrs for the microcosms fertilized at 50 kg/10,000 m<sup>3</sup>/day rabbit excreta loading rate. Each value is an average of bottom and water layers.

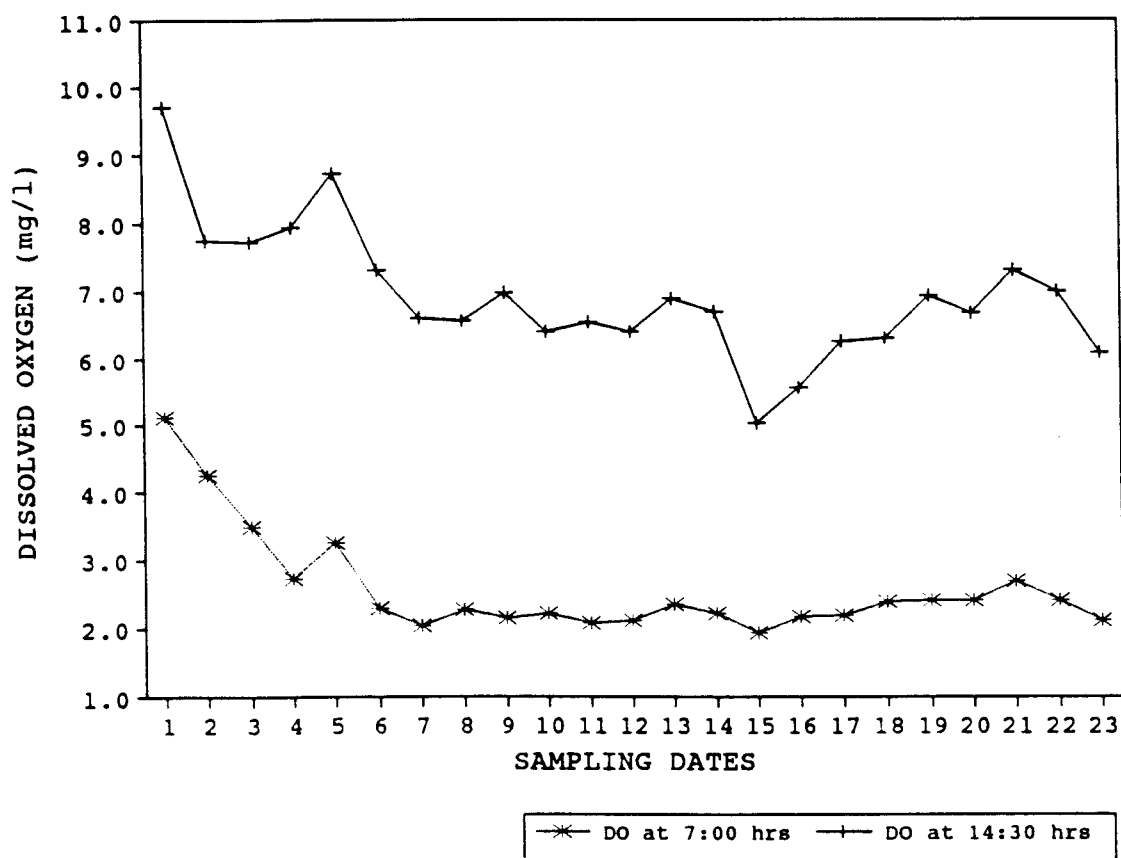


Figure 12. Weekly mean dissolved oxygen concentration at 07:00 and 14:30 hrs for the microcosms fertilized at 75 kg/10,000 m<sup>3</sup>/day rabbit excreta loading rate. Each value is an average of bottom and water layers.

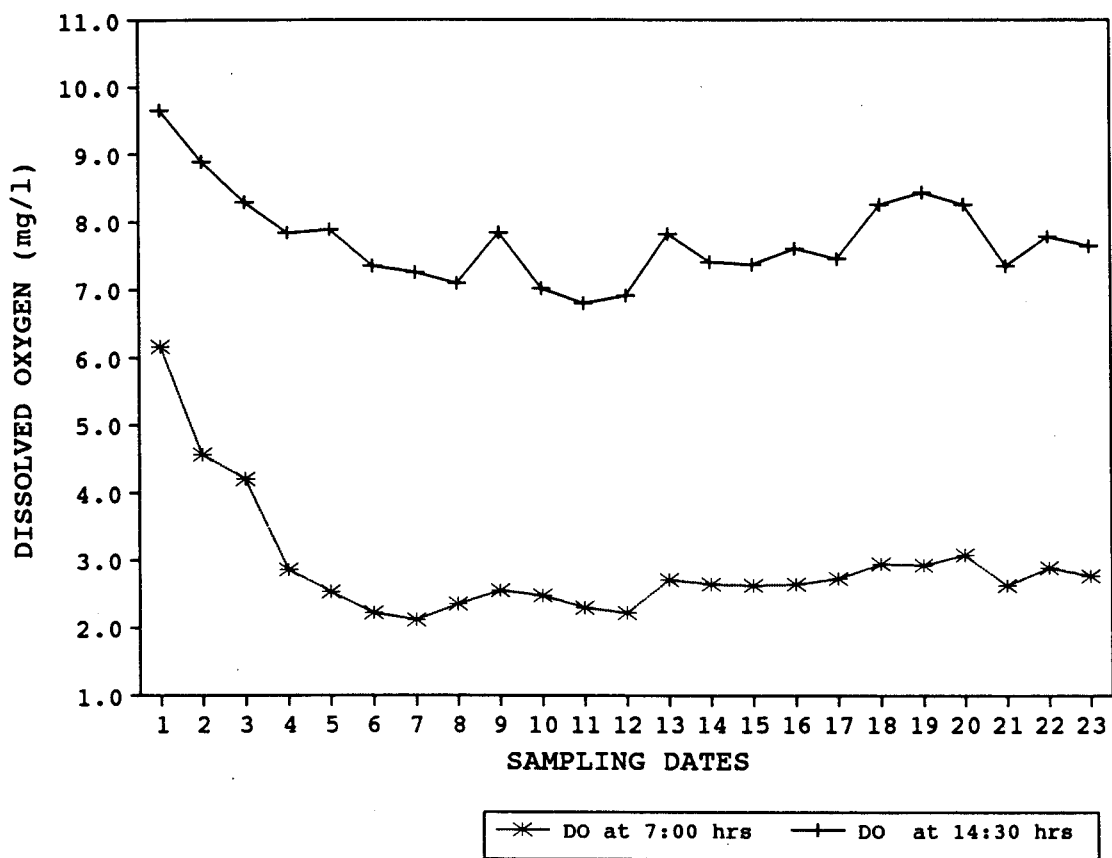


Figure 13. Weekly mean dissolved oxygen concentration at 07:00 and 14:30 hrs for the microcosms fertilized according to the Pondclass computer program. Each value is an average of bottom and surface water layers.