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# Exploring the trophic structure in organically fertilized and feed-driven tilapia culture environments using multivariate analyses

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## Abstract

Reports of similar yields in manure and feed-driven tilapia culture environments raise questions on food utilization in these environments. The possibility that similar production rates are because of utilization of different foods was investigated using exploratory techniques of multivariate analyses. Using factor analysis, trophic pathways through which food becomes available to fish were explored, and using ANOVA models, water quality, sediment quality and tilapia growth and yields were compared. Conceptual graphic models of the main ecological processes occurring in feed-driven and organically fertilized environments are presented and discussed. In both environments, autotrophic and heterotrophic pathways are important processes that result in the availability of natural foods that are utilized by the fish. Extrapolated fish yield data indicate that with equal nutrient input and stocking density, organically fertilized environments could achieve production rates similar to those in feed-driven environments. The general assumption that supplemental or complete foods are well utilized by tilapia in outdoor stagnant ponds remains challenged, and further research on tilapia feeding behaviour and food selection in feed-and organic fertilizer-driven environments is needed.

**Keywords:** organically fertilized environments, feed-driven environments, trophic structure, factor analysis, tilapia production

## Introduction

Tilapia is farmed in varying environments that, based on density and corresponding required addition of inputs, have been classified into extensive, semi-intensive and intensive systems (Edwards 1988; Egna & Boyd 1997). In extensive systems, fish are reared at low densities in stagnant outdoor ponds generally with no inputs at all (Edwards 1988), relying on natural foods for their nutrition. In intensive systems, fish are reared at high densities and depend on nutritionally complete feeds with little or no nutrition from natural foods (Egna & Boyd 1997). In semi-intensive systems, fish are reared in outdoor stagnant ponds, but rely on fertilization to enhance natural food production and/or on supplemental or complete feed to complement the natural foods.

Besides being regarded as better-quality inputs than organic fertilizers, supplemental and complete foods are said to increase food availability in ponds above levels available in organically fertilized ponds. Further, the increased food availability increases fish growth and allows culture of fish at higher stocking densities, resulting in fish yields higher than those

 

possible with organic fertilization (Diana, Lin & Schneeberger 1991; Diana, Lin & Jaiyen 1994). Several researchers reported yields of male tilapia monoculture ranging between 8.6–19.2, 23.7–33 and 30.8–49.4 kg ha$^{-1}$ day$^{-1}$ in manure-only, manure plus supplemental feed and feed-only treatments respectively (Collis & Smitherman 1978; Nerrie 1979; Stone 1980; Hopkins & Cruz 1982; Green, Phelps & Alvarenga 1989; Peralta & Teichert-Coddington 1989; Diana *et al.* 1994). However, tilapia yields comparable with those obtained in feed-only ponds (29.5–30.5 kg ha$^{-1}$ day$^{-1}$) have been reported in manure-only systems (Schroeder, Wohlfarth, Alkon, Halevy & Krueger 1990; Delincé 1992; Knud-hansen, Batterson & Mcnabb 1993).

What fish eat in stagnant outdoor ponds, and hence what drives fish growth and production are still not well understood. If fish in feed-driven environments utilize both natural foods and supplemental feeds, why is it possible to achieve comparable production with organic fertilization only? It can be hypothesized that fish in feed-driven environments reduce their dependency on natural foods. If different foods are used, most likely differences in food webs of organically fertilized and feed-driven environments can be observed. The flow of matter and energy through the food web will influence water quality and sediment properties differently and differences may be observed in the water and sediment properties of organically fertilized and feed-driven ponds.

The goal of this preliminary study was to investigate whether different foods are utilized by tilapia in fertilized and feed-driven environments through multivariate analyses. Using factor analysis, the trophic structures through which food becomes available to the fish were explored and compared. Using ANOVA models, water and sediment qualities in the two environments were compared to establish whether differences because of utilization of different food can be detected on water and pond sediments' qualities. Fish growth rates and yields in the two environments were also compared to establish whether fish performance is better in feed -driven than in organically fertilized environments.

## Materials and methods

### Experimental design

The study was conducted between June and October 2002 at the World Fish Centre, Egypt. Nine newly constructed earthen ponds with concrete banks, 200 m$^2$ and 1.2 m deep, were used for the study. Ponds were filled with water to a 1 m level a week before they were stocked with 18–20 g tilapia (*Oreochromis niloticus*) fingerlings, and water losses because of seepage and evaporation were replaced weekly during the culture period. In a completely randomized design, the ponds were allocated to three treatments in triplicate. In the first treatment, fish were fed 25% protein floating pellets (P) at 3% body weight day$^{-1}$. Ponds in the second treatment were fertilized with chicken manure (C) while in the third, they were fertilized with field grass (G) that was partially composted aerobically for 1 month before pond application.

As the common practice is to have a higher stocking density in feed-driven environments than in organically fertilized environments, ponds in P were stocked at a rate of 2 fish m$^{-2}$ while those in C and G were stocked at a rate of 1 fish m$^{-2}$. To enable a comparison of the systems with different stocking densities, the quantity of chicken manure and composted grass inputs were determined so that nitrogen (N) input rates were half of those in the P treatment. To estimate N input in the P treatment, it was assumed fish would grow to 250 g in 5 months. Assuming a feed conversion ratio of 2% and 16% N in 25% protein pellets, about 7.5 kg of N was required in each P treatment pond. All chicken manure required during the culture period was bought in one batch at the beginning of the experiment. A composite sample was collected and analysed for its nitrogen content, which was found to be 2.5%. Thus, for the same period, chicken manure fertilization rates of about 50 kg ha$^{-1}$ day$^{-1}$ would result in addition of about 4 kg N. After composting, the nitrogen content of the grass was analysed (averaged 2.3%) and applied to the ponds in quantities to be iso-nitrogenous with treatment C. In total, the quantity of composted grass added translated to an average application rate of 60 kg dry matter ha$^{-1}$ day$^{-1}$.

A delay in starting the experiments resulted in a 139-day culture period rather than the originally planned 150-day culture period. In addition, during the culture period, feeding and fertilization were occasionally suspended because of low dawn oxygen levels. The combined effects resulted in a deviation of the originally planned N-loading per treatment. By the end of the culture period, total N input per pond was 5.8, 2.6 and 2.4 in P, C and G respectively. Hence, the N load in organic fertilizer ponds was still about half that in feed-driven ponds.

  

### Fish feeding/pond fertilization and fish growth measurement

Pellets were supplied twice a day at 10:00 and 15:00 hours while chicken manure was applied daily at 10:00 hours and grass compost weekly (every Sunday at 10:00 hours). Fish were sampled monthly using a seine net. At each sampling time, a minimum of 10% of the stocked fish population was seined, counted and weighed (total weight) to calculate the average weight. Feed amounts were adjusted monthly based on the observed average weights and on the assumption of 100% survival. Fertilization or feeding was suspended when dawn oxygen levels dropped below 2 mg $L^{-1}$, and resumed when the dawn oxygen levels were restored above this level. During the last months of culture, it was observed that the fish did not finish their daily feed ratio and from then onwords, they were fed *ad libitum* at the same times of the day as when fed at 3% body weight. At harvesting, fish were seined and the remaining ones were collected by hand from the mud after complete drainage. All fish from a pond were counted, their total weight was recorded and the average weight was determined. Based on these measurements, further calculations of daily fish growth rates and yields were made.

### Water quality sampling and analytical procedures

Temperature, dissolved oxygen (DO) and pH were measured twice daily (6:00 and 15:00 hours), water transparency once daily (12:00 hours) and primary productivity (PP) biweekly. Temperature and DO were measured using an oxygen meter with a combined oxygen and temperature probe (OXYGUARD HANDY III, Oxyguard International, Birkerod, Denmark), pH using a pH electrode (ACCUMET pH meter 25 Fisher Scientific, Pitsburg, PA, USA) and water transparency using a Secchi disk (Boyd & Tucker 1992). Primary productivity was measured using the free water method (Hall & Moll 1975), in which changes in pond water DO at different depths (5, 25, 50 and 75 cm) were monitored over a 24-h period (4 hourly beginning at 06:00 hours). Community respiration is taken as twice the decrease in the mean DO between 18:00 and 06:00 hours the following day. Gross PP is calculated as the sum of gains in the mean DO during the day plus half of community respiration (assumes no difference between diurnal and nocturnal community respiration). Dissolved oxygen values can be corrected for diffusion across the air–water interface by relating the oxygen transfer coefficient to wind speed (Boyd & Teichert-Coddington 1992). However, in this study a correction for diffusion was not made because of unavailability of weather data. The aim of this study was to compare treatments, and as diffusion was the same in all ponds, lack of a diffusion correction does not affect the conclusions of the study.

All other water quality parameters mentioned below were determined biweekly. The first sampling was performed after pond filling and before fish stocking to determine the initial pond water characteristics. Samples were collected at 08:00 hours from three points in a pond using a column sampler (Boyd & Tucker 1992). Samples from the three points were mixed together, a 1L sample was collected from the homogenously mixed composite sample and taken to the laboratory for the various analyses. Soluble reactive phosphorus (SRP) was determined by the ascorbic acid method (APHA 1995) and total phosphorus (TP) by persulphate digestion (Gross & Boyd 1998), followed by the ascorbic acid method. Determination of total ammonia nitrogen (TAN) was performed by the phenate method (APHA 1995), nitrite nitrogen ($NO_2$-N) by the diazotization method (Boyd & Tucker 1992), nitrate nitrogen ($NO_3$-N) by the phenoldisulphinic acid method (Boyd 1979) and total nitrogen (TN) by persulphate digestion (Gross & Boyd 1998), followed by the phenoldisulphinic acid method. Potassium (K) was determined by atomic absorption (Page, Miller & Keeney 1982) and chlorophyll *a* by filtration through GF/C Whatman glass fibre filters followed by acetone extraction and calorimetric determination of the pigment concentration (APHA 1995).

### Sediment samples

Sediment samples were taken from the upper 10cm stratum using a core sampler (Boyd & Tucker 1992). Initial samples were collected before stocking and proceeding samples biweekly. In each pond, nine cores were taken and mixed to form a composite sample from which a sub-sample was taken for analysis. They were analysed for total nitrogen by the Kjeldahl method (Page *et al.* 1982), phosphorus by Olsen's method (Buurman, Lagen & Velthorst 1996), organic carbon by the Walkley–Black dichromate method (Buurman *et al.* 1996) and potassium by determining the exchangeable potassium in a cation exchange-replacing solution ($BaCl_2$), followed by atomic absorption determination of potassium (Buurman *et al.* 1996).

### Statistical analyses

Data were analysed using factor analysis (e.g. Kim & Mueller 1978; Milstein 1993) to identify the main ecological processes acting in the environments. Factor analysis, a multivariate statistical technique, is used to study patterns of interrelationships within one set of variables. The method is based on the analysis of linear relationships, which express the simplest relationships between variables, with a common objective of reducing the number of variables into a smaller number of new hypothetical variables (factors). Factors have no units and are normally distributed standardized variables. The value of each factor for each observation of the original variables can be calculated and used as a new variable in plots, histograms and statistical analyses such as ANOVA. To run the analysis, a data matrix is constructed, with each column containing a variable and each line containing an observation. From the correlation matrix among the variables of that matrix, factors are then extracted by calculating eigenvalues and eigenvectors. The proportion of variance accounted for by each factor is calculated from the corresponding eigenvalue. Each eigenvector contains coefficients of the linear combination corresponding to each original variable. Factors with eigenvalues larger than one are used for interpretation. The interpretation of the factor (hypothetical variable) is then performed based on the relative size and sign of the coefficients (eigenvector). Only coefficients with the highest values are considered for interpretation, usually those larger than 0.5. Of the available techniques for extracting factors, principal component analysis (PCA) (e.g. Seal 1964; Jeffers 1978) was used. The first calculated factor is the linear combination that accounts for as much of the variation contained in the sample as possible. The second factor is the second linear function that accounts for most of the remaining variability, and so on. Factor analysis assumes that observed variables are linear combinations of some underlying factors, independent of one another, which generally reflect an ecological or operational process. Factor analysis is used as an exploratory technique; thus, the results are interpreted as general trends.

After identification, the factors, their differences between treatments and sampling dates as well as the water quality, fish and soil data were compared in a repeated-measures split-plot ANOVA as shown in the model below

$$Y_{ijk} = \mu + \alpha_i + e_{ij} + T_k + (\alpha T)_{ik} + e_{ijk}$$

where $Y_{ijk}$ is the observed value; $\mu$ is the overall mean; $\alpha_I$ are the treatment effects ($i = 3$); $e_{ij}$ is the experimental error because of treatment ($j = 3$); $T_k$ are the time effects ($k = 10$); $(\alpha T)_{ik}$ are the time × treatment cross-effects; and $e_{ijk}$ is the experimental error because of treatment × time interactions. Treatment means were compared using Fisher's least significance difference (LSD) procedure for multicomparison tests (Zar 1984). The analyses were run using the procedures FACTOR and GLM of the SAS statistical package (version 8.2, SAS institute, Cary, NC, USA). Differences were declared significant at an $\alpha$ level of 0.05.

## Results

### Fish growth and yields

Fish growth rates and yields are shown in Table 1. Because of bird predation, survival rates were low and ranged between 41% and 54% with no significant treatment effects. The daily fish growth rate was somewhat higher in C, but differences were not significant among treatments. Average tilapia weights at harvest were significantly higher in C than in P and G but the net fish yield (NFY) was significantly higher in P.

### Water and sediment quality

Results of ANOVA and multi-comparison tests of treatment means of water and sediment quality variables

**Table 1** Tilapia growth and survival rates

| | Treatment | | |
|---|---|---|---|
| **Parameter** | **C** | **G** | **P** |
| Stocking weight (g) | 18.5[a] | 18.3[a] | 19[a] |
| Number stocked | 200 | 200 | 400 |
| Average weight at harvest (g) | 252[a] | 172[b] | 174[b] |
| Survival (%) | 41[a] | 48[a] | 54[a] |
| Daily growth rate (g day$^{-1}$) | 1.7[a] | 1.1[a] | 1.1[a] |
| Total biomass at harvest (kg pond$^{-1}$) | | | |
| Stocked tilapia | 20.7[b] | 16.5[b] | 37.6[a] |
| Recruits | 10.0[a] | 9.5[a] | 15.5[a] |
| Total (stocked tilapia+recruits) | 30.7[b] | 26.0[b] | 53.1[a] |
| Net fish yields (NFY) (recruits inclusive) | | | |
| kg pond$^{-1}$ | 27.0[b] | 22.3[b] | 45.5[a] |
| kg ha$^{-1}$ | 1350[b] | 1117[b] | 2275[a] |
| kg ha$^{-1}$ year$^{-1}$ (assuming two seasons) | 2700[b] | 2234[b] | 4550[a] |

Values are means of three replicates for each treatment. Same letters (superscripts) indicate no significant difference at the 0.05 level. a > b > ···.

  

**Table 2** ANOVA and multi-comparison of treatment means (by Fishers' least significance difference test (LSD)) of variables measured daily

| Variables | Dissolved oxygen (mg $L^{-1}$) (06:00 hours) | | Dissolved oxygen (mg $L^{-1}$) (15:00 hours) | | Temperature (°C) (06:00 hours) | | Temperature (°C) (15:00 hours) | | Secchi depths (cm) (12:00 hours) | | pH (06:00 hours) | | pH (15:00 hours) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *ANOVA models* | | | | | | | | | | | | | | |
| Significance | *** | | *** | | *** | | *** | | *** | | *** | | *** | |
| Coeff. determination ($r^2$) | 0.92 | | 0.85 | | 0.99 | | 0.99 | | 0.72 | | 0.91 | | 0.87 | |
| **Variance source** | **Sign.** | **%SS** | **Sign.** | **%SS** | **Sign.** | **%SS** | **Sign.** | **%SS** | **Sign.** | **%SS** | **Sign.** | **%SS** | **Sign.** | **%SS** |
| TRT | NS | 0 | *** | 36 | * | 0 | NS | 0 | * | 27 | *** | 62 | *** | 56 |
| WEEK | *** | 86 | *** | 53 | *** | 99 | *** | 99 | *** | 40 | *** | 10 | *** | 23 |
| WEEK × TRT | *** | 14 | *** | 11 | *** | 1 | NS | 1 | *** | 33 | *** | 28 | *** | 21 |
| **Multi-comparison of means by TRT** | | | | | | | | | | | | | | |
| C | 1.5 a | | 12.3 a | | 26.3 ba | | 29.4 a | | 14.4 ba | | 9.1 a | | 9.3 a | |
| G | 1.4 a | | 10.3 b | | 26.2 b | | 29.3 a | | 16.9 a | | 8.8 b | | 9.1 b | |
| P | 1.4 a | | 7.6 c | | 26.5 a | | 29.2 a | | 12.3 b | | 8.3 c | | 8.6 c | |

*Significant at the 0.05 level.
***Significant at the 0.001 level.
Same letters in the multi-comparison of means columns indicate no significant difference at the 0.05 level. a > b > ···.
Coeff., coefficient; Sign., significance level: NS, not significant; %SS, percent of total sum of squares; TRT, treatment.

are shown in Tables 2 and 3. The model accounted for over 70% of the variability in all the variables ($r^2$ of the ANOVA), and the main source of variability in water and sediment variables was week, which represents the time effect.

## Factor analysis

Results of the factor analysis are presented in Table 4. Five factors were important in describing the variability of the data and together accounted for 74% of the overall data variability. The five factors (F1–F5) are identified and described in the paragraphs below, and results of ANOVA and multi-comparison tests of the factors' means by treatment and time are shown in Table 5.

### *Factor 1 (F1): phytoplankton biomass synthesis in the water column*

This first factor accounted for 30% of the data variability (Table 4). It comprises two groups of variables: those with high positive coefficients and those with high negative coefficients. High factor values indicate high PP, chlorophyll *a* levels, organic phosphorus and organic nitrogen, monthly average fish weights, $NO_2$-N and $NO_3$-N and K in both sediment and water column (variables with positive coefficients), together with low morning DO, afternoon temperature and Secchi depth transparency (variables with negative coefficients) (Table 4 – F1). The factor reflects phytoplankton biomass synthesis in the water column. High PP levels reflect high rates of phytoplankton synthesis, while high values in chlorophyll *a*, organic nitrogen and phosphorus reflect the presence of high plankton biomass in the water column. The high synthesis of phytoplankton biomass is a result of availability of inorganic nutrients (Hargreaves 1998) in the water (high positive values in $NO_2$-N and $NO_3$-N). Phytoplankton biomass supports fish growth (Diana *et al.* 1991) but also increases water turbidity resulting in low Secchi depth transparency. Night respiration of the high phytoplankton biomass, coupled with other biotic respiration, consumes oxygen, resulting in low values of DO in the morning. In the ANOVA and mean multi-comparison test of the factor (Table 5), the applied model accounted for 97% of the factors' variability, of which 86% was owing to time (weeks), 9% owing to treatment and 5% owing to the interaction of treatment and time. The multi-comparison of the factor's means by week shows that biomass increased with time, and the multi-comparison of means by treatment shows that the highest plankton biomass occurred in treatment C followed

**Table 3** ANOVA and multi-comparison of means (LSD) of variables measured weekly

| Variables | Total ammonia nitrogen in pond water (mg L$^{-1}$) | | $NO_2$-N (in pond water) (mg L$^{-1}$) | | $NO_3$-N (in pond water) (mg L$^{-1}$) | | Total nitrogen (in pond water) (mg L$^{-1}$) | | Soluble phosphorus (in pond water) (mg L$^{-1}$) | | Total phosphorus (in pond water) (mg L$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ANOVA models | | | | | | | | | | | | |
| Significance | *** | | *** | | *** | | *** | | *** | | *** | |
| Coeff. determination ($r^2$) | 0.77 | | 0.96 | | 0.91 | | 0.82 | | 0.95 | | 0.84 | |
| **Variance source** | **Sign.** | **%SS** | **Sign.** | **%SS** | **Sign.** | **%SS** | **Sign.** | **%SS** | **Sign.** | **%SS** | **Sign.** | **%SS** |
| TRT | NS | 3 | ** | 7 | *** | 14 | * | 13 | ** | 16 | ** | 21 |
| WEEK | *** | 83 | *** | 64 | *** | 76 | *** | 70 | *** | 66 | *** | 60 |
| WEEK × TRT | NS | 15 | *** | 29 | ** | 9 | * | 17 | *** | 18 | ** | 19 |
| **Multi-comparison of means by TRT** | | | | | | | | | | | | |
| C | 0.54 a | | 0.04 b | | 0.13 b | | 11.9 a | | 0.11 a | | 1.05 a | |
| G | 0.47 a | | 0.07 a | | 0.21 a | | 9.5 ba | | 0.05 b | | 0.79 b | |
| P | 0.45 a | | 0.009 c | | 0.10 c | | 7.9 b | | 0.03 b | | 0.71 b | |
| **Multi-comparison of means by WEEK** | | | | | | | | | | | | |
| 1 | 0.4 cb | | 0.006 b | | 0.06 g | | 4.2 e | | 0.06 cb | | 0.38 c | |
| 2 | 0.8 a | | 0.001 b | | 0.09 gfe | | 4.4 e | | 0.06 b | | 0.40 c | |
| 3 | 0.4 cb | | 0.010 b | | 0.08 gf | | 7.1 d | | 0.01 e | | 0.72 b | |
| 4 | 0.3 c | | 0.009 b | | 0.12 edcb | | 7.5 d | | 0.07 b | | 0.90 a | |
| 5 | 0.7 a | | 0.001 b | | 0.12 fedc | | 12.3 b | | 0.03 e | | 1.01 a | |
| 6 | 0.4 cb | | 0.001 b | | 0.11 fed | | 11.9 b | | 0.03 e | | 0.99 a | |
| 7 | 0.1 d | | 0.000 b | | 0.15 cb | | 12.6 b | | 0.03 ed | | 0.99 a | |
| 8 | 0.7 a | | 0.000 b | | 0.15 dcb | | 10.8 cb | | 0.04 ed | | 1.00 a | |
| 9 | 0.7 a | | 0.180 a | | 0.40 a | | 17.5 a | | 0.05 dcb | | 1.06 a | |
| 10 | 0.5 b | | 0.180 a | | 0.18 b | | 9.1 dc | | 0.27 a | | 1.02 a | |

| Variables | Potassium (in pond water) (mg L$^{-1}$) | | Chlorophyll *a* (mg L$^{-1}$) | | Primary production (mg $O_2$ L$^{-1}$) | | Available phosphorus (in sediment) (%) | | Total nitrogen (in sediment) (%) | | Potassium (in sediment) (%) | | Organic carbon (in sediment) (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ANOVA models | | | | | | | | | | | | | | |
| Significance | *** | | *** | | *** | | *** | | *** | | *** | | *** | |
| Coeff. determination ($r^2$) | 0.93 | | 0.86 | | 0.93 | | 0.64 | | 0.69 | | 0.98 | | 0.82 | |
| **Variance source** | **Sign.** | **%SS** | **Sign.** | **%SS** | **Sign.** | **%SS** | **Sign.** | **%SS** | **Sign.** | **%SS** | **Sign.** | **%SS** | **Sign.** | **%SS** |
| TRT | NS | 2 | *** | 22 | *** | 24 | NS | 12 | NS | 16 | NS | 1 | NS | 39 |
| WEEK | *** | 92 | *** | 66 | *** | 65 | *** | 91 | * | 34 | *** | 98 | *** | 36 |
| WEEK × TRT | ** | 6 | ** | 22 | *** | 11 | NS | 8 | NS | 50 | NS | 1 | NS | 25 |
| **Mean multi-comparison by TRT** | | | | | | | | | | | | | | |
| C | 13.8 a | | 381 a | | 20.1 a | | 0.18 a | | 0.15 a | | 0.07 a | | 1.7 a | |
| G | 14.8 a | | 264 b | | 16.1 b | | 0.17 a | | 0.12 a | | 0.07 a | | 1.3 a | |
| P | 14.3 a | | 211 c | | 11.9 c | | 0.17 a | | 0.14 a | | 0.07 a | | 1.7 a | |
| **Mean multi-comparison by WEEK** | | | | | | | | | | | | | | |
| 1 | 8.90 g | | 17 f | | 5.01 e | | 0.28 a | | 0.12 cb | | 0.059 ed | | 1.9 a | |
| 2 | 11.8 f | | 123 e | | 7.20 ed | | 0.16 cb | | 0.12 cb | | 0.057 e | | 1.5 dc | |
| 3 | 9.3 g | | 241 d | | 15.4 c | | 0.14 dcb | | 0.11 c | | 0.060 d | | 1.4 dc | |
| 4 | 16.7 cb | | 300 dc | | 18.3 b | | 0.12 dc | | 0.13 cba | | 0.065 c | | 1.2 d | |
| 5 | 14.8 e | | 348 cb | | 22.1 a | | 0.11 dc | | 0.13 cba | | 0.084 b | | 1.4 dc | |
| 6 | 18.3 a | | 316 c | | 21.0 a | | 0.14 dcb | | 0.13 cba | | 0.055 f | | 1.4 dc | |
| 7 | 16.1 dc | | 347 cb | | 18.8 b | | 0.27 a | | 0.15 a | | 0.060 d | | 1.5 c | |
| 8 | 17.4 ba | | 400 ba | | 21.0 a | | 0.15 dcb | | 0.14 ba | | 0.088 a | | 1.6 cb | |
| 9 | 15.2 ed | | 462 a | | 15.0 c | | 0.08 d | | 0.15 a | | 0.088 a | | 1.8 ba | |
| 10 | 14.2 e | | 298 dc | | 16.6 cb | | 0.21 ba | | 0.14 ba | | 0.082 b | | 1.7 cba | |

*Significant at the 0.05 level.

**Significant at the 0.01 level.

***Significant at the 0.001 level.

Same letters in the multi-comparison of means columns indicate no significant difference at the 0.05 level. a > b > ···.

Coeff., coefficient; Sign. = significance level; NS, not significant; %SS, percent of total sum of squares; TRT, treatment.

  

**Table 4** Factor analysis results

| Factors | F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|---|
| Morning dissolved oxygen (DO) | **−0.68** | −0.28 | 0.42 | 0.15 | 0.13 |
| Afternoon dissolved oxygen | 0.42 | **0.64** | 0.15 | −0.05 | −0.13 |
| Morning temperature | −0.45 | **0.74** | −0.26 | 0.14 | 0.18 |
| Afternoon temperature | **−0.55** | **0.74** | −0.16 | 0.15 | 0.15 |
| Morning pH | 0.24 | **0.52** | **0.73** | 0.01 | 0.21 |
| Afternoon pH | 0.32 | **0.71** | **0.53** | 0.08 | 0.21 |
| Secchi depth | **−0.50** | −0.23 | 0.29 | −0.21 | 0.00 |
| Total ammonia nitrogen (TAN) | 0.02 | −0.28 | −0.21 | 0.35 | **0.54** |
| Nitrite nitrogen ($NO_2^-$ N) | **0.50** | **−0.57** | 0.25 | −0.28 | 0.11 |
| Nitrate nitrogen ($NO_3^-$ N) | **0.58** | −0.35 | −0.01 | −0.30 | 0.30 |
| Organic nitrogen | **0.77** | 0.20 | 0.07 | −0.02 | 0.04 |
| Soluble reactive phosphorus | 0.27 | −0.39 | **0.56** | 0.08 | −0.20 |
| Organic phosphorus | **0.77** | 0.42 | −0.06 | 0.05 | 0.01 |
| Potassium (K) | **0.56** | 0.22 | −0.41 | −0.14 | −0.28 |
| Chlorophyl *a* | **0.87** | 0.32 | 0.05 | 0.09 | 0.12 |
| Primary productivity (PP) | **0.71** | **0.59** | 0.03 | 0.02 | −0.01 |
| Monthly average fish weight | **0.89** | −0.11 | 0.04 | −0.02 | −0.18 |
| Sediment phosphorus | −0.04 | 0.17 | 0.41 | −0.10 | **−0.68** |
| Sediment nitrogen | 0.4 | −0.15 | 0.10 | **0.70** | −0.21 |
| Sediment potassium | **0.68** | −0.36 | −0.16 | 0.22 | 0.29 |
| Sediment organic carbon | 0.04 | −0.37 | 0.06 | **0.72** | −0.20 |
| Variance explained (%) | 30 | 20 | 11 | 7 | 6 |
| Interpretation | Phytoplankton biomass synthesis in the water column | Photosynthetic activity | Role of pH in orthophosphate liberation | Organic matter accumulation in the sediment | Organic matter decomposition |

Coefficients in bold were used for interpretation.

by G and the lowest in P (Table 5 – F1). Figure 1 – F1 illustrates the factor's trend over time in the three treatments (week × treatment interaction effect). It shows similar biomass in the first 2 weeks in all treatments, and from then on the biomass increase was larger in C than in G, the latter being larger than in P.

*Factor 2 (F2): photosynthetic activity in the water column*

This factor accounted for a further 20% of the data variability (Table 4). It has high positive coefficients in temperature, pH, DO in the afternoon hours and PP, and a high negative coefficient in $NO_2$-N (Table 4 – F2). It reflects photosynthetic activity in the water column. High temperatures favour high photosynthetic activity in which primary producers utilize carbon dioxide and release oxygen. Reduction in carbon dioxide in the water leads to an increase of water pH (Boyd 1998). Released oxygen is utilized in the nitrification processes, and hence reduction in $NO_2$-N (Boyd 1998). The ANOVA model accounted for 97% of the factors' variability, of which 84% was because of time, 11% because of treatment and 6% because of the interaction between time and treatment. Multi-comparison tests of means by week show that photosynthetic activity increased with time in the first months but decreased in the last 2 months, while multi-comparison of treatment means shows that photosynthetic activity was significantly higher in C than in G than P (Table 5 – F2). The cross-effect of treatment and time (Fig. 1 – F2) shows that photosynthetic activity in the first 2 and last 2 weeks was similar in all treatments, while in between was higher in C than in G than in P.

*Factor 3 (F3): role of pH in orthophosphate liberation*

A further 11% of the overall data variability is accounted for by this factor. It shows high positive coefficients in water pH and SRP in the water column (Table 4 – F3). It reflects the role of pH in orthophosphate liberation. An increase of pH causes the dissolution of phosphate, resulting in an increase of orhophosphate concentration in the water column (Kamp-Nielson 1974; Boyd 1990). Besides this dissolution, an increase of pH will make the surface charge of particles (hydroxides and clays) more negative, causing a lesser ability to adsorb the negative phosphate ions (Hillerod 1974) that then remain in the water col-

**Table 5** ANOVA of factors and multi-comparison test (LSD) of their treatment means

| **Factors:** | **F1** | | **F2** | | **F3** | | **F4** | | **F5** | |
|---|---|---|---|---|---|---|---|---|---|---|
| *ANOVA models* | | | | | | | | | | |
| Significance | *** | | *** | | *** | | *** | | *** | |
| Coeff. determination ($r^2$) | 0.97 | | 0.97 | | 0.94 | | 0.83 | | 0.85 | |
| **Variance source** | **Sign.** | **%SS** | **Sign.** | **%SS** | **Sign.** | **%SS** | **Sign.** | **%SS** | **Sign.** | **%SS** |
| TRT | *** | 8.8 | ** | 10.7 | *** | 48.6 | NS | 51.9 | * | 9.8 |
| WEEK | *** | 86 | *** | 83.7 | *** | 32.2 | *** | 25.0 | *** | 81.9 |
| WEEK × TRT | *** | 5.2 | *** | 5.6 | *** | 19.2 | * | 23.1 | NS | 8.3 |
| **Multi-comparison of means by TRT** | | | | | | | | | | |
| C | 0.40 | a | 0.40 | a | 0.8 | a | 0.4 | a | −0.06 | ba |
| G | −0.05 | b | 0.01 | b | 0.1 | b | −0.7 | a | 0.4 | a |
| P | −0.30 | c | −0.4 | c | −0.9 | c | 0.3 | a | −0.30 | b |
| **Multi-comparison of means by WEEK** | | | | | | | | | | |
| 1 | −1.55 | f | −0.8 | g | 1.1 | a | 0.2 | cba | −0.3 | d |
| 2 | −1.40 | f | −0.4 | f | 0.1 | dc | 0.3 | ba | −0.5 | cb |
| 3 | −0.60 | e | 0.7 | cb | 0.4 | cb | −0.03 | dcb | 0.7 | ba |
| 4 | −0.30 | d | 0.9 | ba | −0.3 | f | −0.2 | dc | 0.2 | dc |
| 5 | 0.20 | c | 0.6 | dc | −0.8 | g | 0.6 | a | 0.9 | ba |
| 6 | 0.30 | c | 0.4 | a | −0.5 | f | −0.4 | dc | −1.3 | e |
| 7 | 0.30 | c | 0.4 | ed | 0.02 | ed | −0.3 | dc | −1.2 | e |
| 8 | 0.70 | b | 0.3 | e | −0.5 | gf | 0.5 | ba | 0.1 | dc |
| 9 | 1.40 | a | −1.3 | h | −0.2 | fe | −0.3 | dc | 1.1 | a |
| 10 | 0.90 | b | −1.5 | i | 0.6 | b | −0.4 | d | −0.8 | e |

*Significant at the 0.05 level.
**Significant at the 0.01 level.
***Significant at the 0.001 level.
Same letters in the mean multi-comparison columns indicate no significant difference at the 0.05 level. a > b > ···.
Sign., significance level: NS, not significant; %SS, percentage of total sum of squares.

umn. The ANOVA model accounted for 94% of this factor variability, of which 49% was owing to treatment, 32% owing to time and 19% owing to the interaction between time and treatment. The multi-comparison of means by treatment show significant treatment effects, with higher means in C than G than P. The multi-comparison of means by week shows that the factor was high in the first weeks, decreased and remained low in the following weeks, increasing again in the last weeks (Table 5 – F3). Figure 1 – F3 shows that in the beginning of the culture period all treatments had similar F3 values, but from the third week on there were strong differences between treatments.

*Factor 4 (F4): accumulation of organic matter in the sediment*

This factor accounted for a further 7% of the data variability. It shows high positive coefficients for organic carbon and nitrogen in the sediment (Table 4 – F4), reflecting accumulation of organic matter. Organic carbon and nitrogen occur in soil as a constituent of organic substances (Boyd 1995) and most of the nitrogen in aquaculture pond sediments is associated with organic matter (Boyd 1990; Hargreaves 1998). The ANOVA model accounted for 83% of this factor variability, of which 25% was owing to time, 23% owing to the interaction between time and treatment, and no significant treatment effect. The multi-comparison of means by week shows a trend of increased organic matter accumulation in the sediment in the first month, with some variations afterwards (Table 5 – F4). In the treatment and week cross-effect (Fig. 1 – F4), variations in time were different in the three treatments.

*Factor 5 (F5): decomposition of organic matter*

It accounted for a further 6% of the data variability. It shows a high positive coefficient in TAN in the water

  

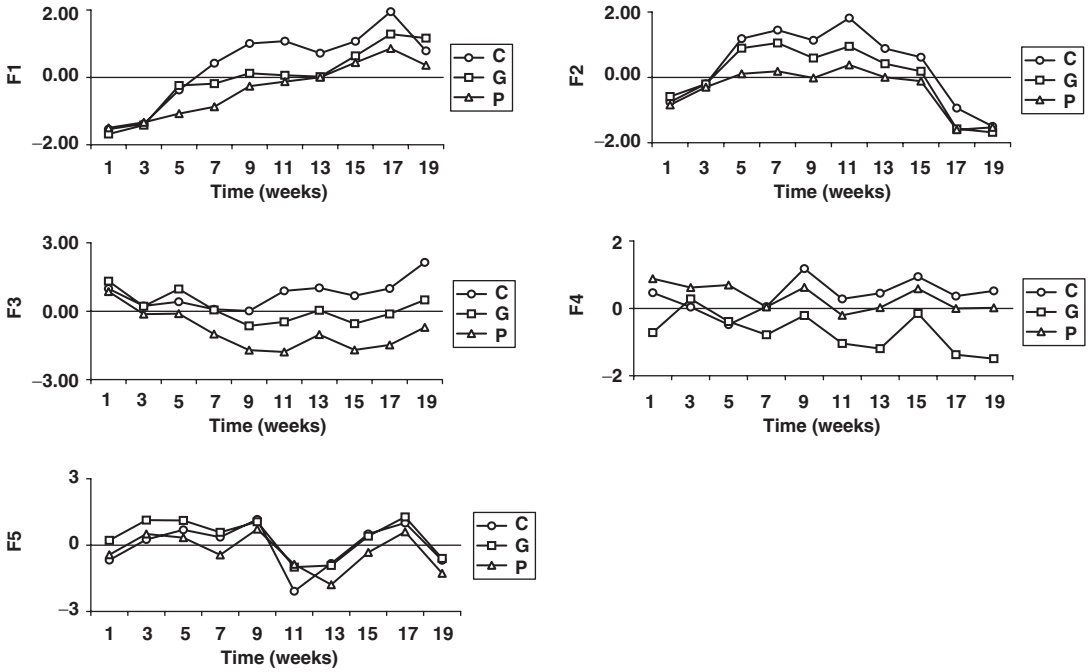


**Figure 1** Treatment and week interaction for factors 1–5.

column and a high negative coefficient in sediment phosphorus (Table 4 – F5). This reflects decomposition of organic matter. During the decomposition of organic matter in sediments, phosphates are released, leading to a decline in sediment phosphorus (Hillerod 1974; De Pinto, Young, Bonner & Rodgers 1986; Boyd 1990) and organic nitrogen is mineralized to ammonia, increasing the TAN in the water column (Boyd 1995). The ANOVA model accounted for 85% of the factors' variability, of which 82% was owing to time and 10% owing to treatment. Multi-comparison of means by week shows that organic matter decomposition increased over time during the first half of the culture period and was lower in the second half. Multi-comparison of means by treatment shows that decomposition in G was significantly higher than in P (Table 5 – F5).

## Discussion

### Fish growth and yields

Similar fish growth rates in all the environments imply that organically fertilized environments can perform equally well as feed-driven environments, or perhaps better considering the significantly higher tilapia average weights at harvest in C (Table 1). Although stocking density in the feed-driven environment was twice that in organically fertilized environments, nutrient inputs were also twice as much. The higher NFY in P is a result of the double stocking density. Assuming equal nutrient input in the environments (double the organic fertilizer quantity), equal stocking density and assuming the observed growth rates in the organic manure environments, NFY in C and G would be double the values in Table 1 and would be comparable with those in P. Similar observations were made by Knud-Hansen *et al.* (1993), who concluded that with increased primary production in organically fertilized environments, food constraints at higher stocking densities can be overcome.

### Ecological processes defining the food webs

Conceptual graphic models of the main ecological processes in the organic fertilized and the feed-driven environments are shown in Figs 2 and 3. The figures were constructed following the results of the factor and ANOVA analyses (Tables 2–4), and the widths of the arrows represent the importance of the flows.

The factors describe autotrophic and heterotrophic pathways in which photosynthetic activities (F2) result in the development of phytoplankton biomass (F1) in the water column. Phytoplankton cells are said to have a lifespan of 1–2 weeks (Boyd 1995),

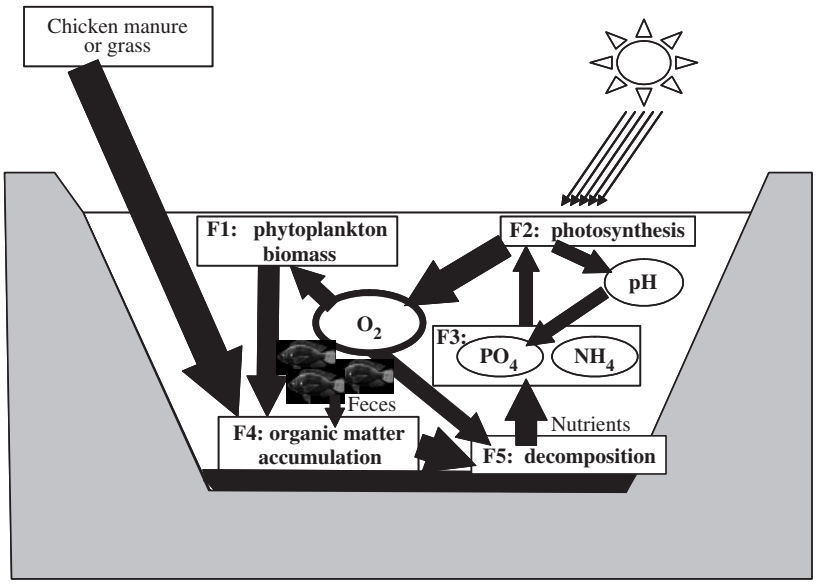


**Figure 2** Main ecological processes occurring in organically fertilized environments.

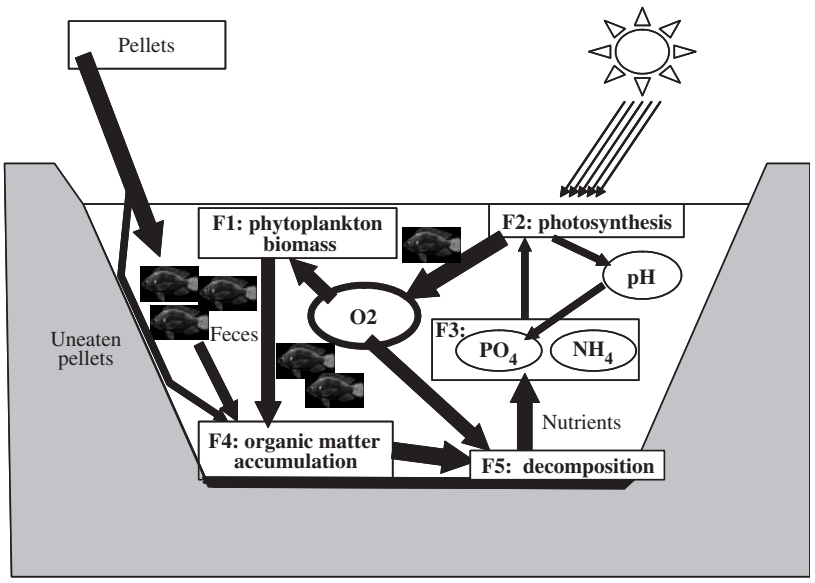


**Figure 3** Main ecological processes occurring in feed-driven environments.

after which dead cells settle at the pond bottom at a daily rate of about 50% of the algal standing crop (Schroeder, Alkon & Laher 1991). This algal matter, in addition to organic matter from pond inputs, accumulates in pond sediment (F4) and supports the heterotrophic pathway by providing a substrate for decomposition (F5). In turn, decomposition favours the liberation of orthophosphate (F3) and a flux of inorganic nitrogen from the sediment (Boyd 1995; Shrestha & Lin 1996). Nutrients released into the water column from decomposition augment the availability of inorganic nutrients (SRP and TAN) for higher rates of phytoplankton synthesis and results in increased algal biomass and more organic matter settling in the sediment. High photosynthetic rates supply the oxygen needed for decomposition and the cycle continues with increasing rates over time, hence the reason why time accounts for a high proportion of variability in most of the factors (Table 4).

The autotrophic and heterotrophic pathways developed in all three treatments, and hence natural food availability. However, except for F4, the magnitude of the factors was higher in the organically fertilized environments (Table 4) and also proceeded at

  

a higher rate in the fertilized than in the feed-driven environments (Fig. 1). In P, the quantity of organic matter input was lower and much so at the beginning when the fish were small. Moreover, only a fraction of the feed remained unconsumed. For example, in the first week, P received 240 g of feed pond $^{-1}$ day $^{-1}$. As only 15% sediments as uneaten feed (Boyd & Tucker 1995) and 30% of the ingested feed is excreted (Porter, Krom, Robbins, Bricknell & Davidson 1987), only about 97 g pond $^{-1}$ day $^{-1}$ would form organic matter at the bottom. On the other hand, C and G received at least 1 kg of organic input (from organic fertilizer) pond $^{-1}$ day $^{-1}$. The higher organic matter input in C and G than in P, especially in the first weeks of culture when feed quantities were low, resulted in higher decomposition rates (F5) during which larger amounts of nutrients were released (F3). For example, assuming 16% N in the 25% protein feed, only 4 g N pond $^{-1}$ day $^{-1}$ would be released upon decomposition of the 97 g organic matter, whereas assuming complete decomposition of 1 kg chicken manure (2.5% N content), 25 g N pond $^{-1}$ day $^{-1}$ would be released in C. Consequently, larger amounts of nutrients would became available for photosynthesis (F2), resulting in higher phytoplankton biomass (F1). Higher organic inputs in C and G did not result in more organic matter accumulation on the bottom (F4), because of efficient microbial processes (Maclean, Brown, Ang & Jauncey 1994), which result in rapid organic decomposition (Smith 1996). Microbes responsible for organic matter decomposition are present in all ponds; their numbers increase with an increase of organic matter (Boyd 1998; Gasol & Duarte 2000) and can process organic matter at the same rate as it is precipitated (Schroeder 1987).

As the culture period progressed, fish weights increased and feed amounts in treatment P were regularly adjusted upwards with the increase of fish weights. With an increase of feed amounts with time, the quantity of organic matter (F4) input from uneaten feed and faecal matter also increases. Expectedly, decomposition rates (F5) and nutrient release (F3) should increase too, approaching levels of those released in C and G, and result in increased rates of photosynthesis (F2) and plankton biomass (F1). However, the rate of the processes still remained lower in P (Fig. 1), which could be explained by the double fish biomass in P. Consumption of natural foods by the higher fish biomass can maintain the phytoplankton biomass lower in P. With a lower phytoplankton biomass, organic matter input from phytoplankton sedimentation would also be lower, maintaing heterotrophic and autotrophic activities lower too. This, on the other hand, implies similar rates of natural food consumption in both feed driven and organically fertilized environments, and disputes the study hypothesis that natural foods are less utilized in feed-driven environments. Similar observations have been reported in previous studies where natural foods accounted for a great percentage (50–80%) of tilapia growth, both in fertilizer and in feed-driven environments (Schroeder 1983; Schroeder *et al.* 1990; Knud-Hansen *et al.* 1993; Lochman & Phillips 1996). The question therefore is whether the pellet feeds are efficiently utilized or are an expensive pond fertilizer (Green, El Nagdy & Hebicha 2002). The results further contribute toward the on-going debate on the merits of supplementary or complete feeds in semi-intensive tilapia culture in the tropics (Green 1992; Shang 1992) where feed costs are a major factor limiting fish production (Omondi, Gichuri & Veverica 2001). Further investigations on food selection in feed and organically fertilized environments could offer useful information on this debate.

## Conclusion

The trophic structure in organically fertilized and fed ponds is similar (Figs 2 and 3), as autotrophic and heterotrophic pathways are important processes in both environments, providing natural foods that are a main part of the diet in both environments. The results support previous indications that with equal nutrient inputs and stocking densities, manure-driven environments could perform equally well as feed-driven environments. Further, they support emerging evidence that supplemental feeds may not be well utilized by fish in outdoor stagnant ponds.

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