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# Effects of season on ecological processes in extensive earthen tilapia ponds in Southeastern Brazil

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(With 2 figures)

## Abstract

In Southeastern Brazil tilapia culture is conducted in extensive and semi-intensive flow-through earthen ponds, being water availability and flow management different in the rainy and dry seasons. In this region lettuce wastes are a potential cheap input for tilapia culture. This study examined the ecological processes developing during the rainy and dry seasons in three extensive flow-through earthen tilapia ponds fertilized with lettuce wastes. Water quality, plankton and sediment parameters were sampled monthly during a year. Factor analysis was used to identify the ecological processes occurring within the ponds and to construct a conceptual graphic model of the pond ecosystem functioning during the rainy and dry seasons. Processes related to nitrogen cycling presented differences between both seasons while processes related to phosphorus cycling did not. Ecological differences among ponds were due to effects of wind protection by surrounding vegetation, organic loading entering, tilapia density and its grazing pressure on zooplankton. Differences in tilapia growth among ponds were related to stocking density and ecological process affecting tilapia food availability and intraspecific competition. Lettuce wastes addition into the ponds did not produce negative effects, thus this practice may be considered a disposal option and a low-cost input source for tilapia, at least at the amounts applied in this study.

*Keywords:* earthen pond ecology, flow-through, seasonal variations, tilapia extensive culture, water quality.

## Efeitos da época sobre os processos ecológicos em viveiros escavados para criação extensiva de tilápias no Sudeste do Brasil

## Resumo

No sudeste do Brasil, a criação extensiva e semi-intensiva de tilápias é realizada em viveiros escavados com fluxo contínuo, com a disponibilidade e manejo do fluxo de água diferentes nas épocas chuvosa e seca. Na região, os resíduos de alface são um recurso potencialmente de baixo custo para a tilapicultura. Este estudo examinou os processos ecológicos ocorrendo em três viveiros escavados com fluxo contínuo fertilizados com restos de alface para criação extensiva de tilápias durante as épocas chuvosa e seca. Parâmetros de qualidade de água, plâncton e sedimento foram amostrados mensalmente durante um ano. A análise de fator foi utilizada para identificar os processos ecológicos nos viveiros e elaborar um modelo gráfico do funcionamento do ecossistema dos viveiros durante as duas épocas. Os processos relacionados ao ciclo do nitrogênio mostraram diferenças entre as duas épocas, enquanto que os processos relacionados com o ciclo do fósforo não foram influenciados pela época. As diferenças ecológicas entre os viveiros foram principalmente devido aos efeitos do vento, adição de material orgânico e densidade de estocagem. Diferenças no crescimento das tilápias entre os viveiros relacionaram-se com a densidade de estocagem e os processos ecológicos afetando a disponibilidade de alimento e a competição intraespecífica. A adição dos restos de alface nos viveiros não resultou em efeitos negativos, assim esta prática pode ser considerada um destino alternativo e um recurso de baixo custo para a criação extensiva de tilápias, pelo menos nas quantidades investigadas neste estudo.

*Palavras-chave:* ecologia de viveiros, fluxo contínuo, variações sazonais, tilapicultura extensiva, qualidade de água.

## 1. Introduction

Extensive and semi-intensive earthen ponds are the main aquaculture systems in Brazil (FAO, 2013). In Southeastern Brazil fish culture is mostly done in flow-through systems using a series of inline earthen fishponds, where the first pond receives water from a natural source and the downstream ones receive the effluents from the upstream ponds. Studies on variability in water quality parameters in such a flow-through semi-intensive polyculture system (inset B of Figure 1, fish density 1-20 fish/m$^2$) showed that ammonia, nitrate, phosphorous, conductivity and chlorophyll-a increased in the downstream ponds, whereas dissolved oxygen and water transparency decreased, leading to fishpond eutrophication (Sipaúba-Tavares et al., 2007, 2010). Those studies also found water quality differences between the dry and rainy seasons, mainly due to cumulative effects of suspended and dissolved materials washed into the fishponds during the rainy season.

Nile tilapia (*Oreochromis niloticus* L.) is the main species cultured in Brazil, accounting for 39% of freshwater fish farming in the country (Brasil, 2012). Its farming is increasing in the country due to the expansion of domestic and foreign markets (Fülber et al., 2009). This activity is mainly practiced by small-scale scattered farmers (Zimmermann, 2008). Nile tilapia feeds on phytoplankton, zooplankton and benthic detritus (Beveridge and Baird, 2000; Cuvin-Aralar, 2003). Since it feeds low in the food chain it is an attractive option for small-scale farmers that can utilize locally available agricultural wastes and by-products as pond inputs (Köprücü and Özdemir, 2005; Gonzales Junior et al., 2007). Vegetables, such as weeds and fresh leaves, as well as swine, cattle, buffalo and chicken manure, are commonly used as fishpond inputs to enrich primary productivity (Flores-Nava, 2007).

In Brazil, lettuce (*Lactuca sativa* L.) is cultured throughout the territory, and is one of the most largely produced and traded leafy greens (Pôrto et al., 2008, 2012). In Southeastern Brazil, lettuce is a major horticulture, mainly produced by small families close to urban areas (Silva et al., 2000; Villas-Bôas et al., 2004), producing large amounts of discarded biomass (Vilela et al., 2003; CEAGESP, 2013) that until now has not been tried as fishpond input. Lettuce has 4.6% nitrogen and 1.2% phosphorus (Hartz and Johnstone, 2007), values higher than those observed in several types of manure (3.7% N in chicken manure, 1.6% N and 0.5% P in cattle and buffalo manure) (Lin et al., 1997; Yi et al., 2004), which makes it potentially more efficient as a fertilizer. Using lettuce wastes as pond fertilizer and food for tilapia may decrease costs for the fish producers and at the same time offer a more sustainable way to discard lettuce waste.

In the flow-through systems of Southeastern Brazil water input strongly depends on seasonal rains. Thus,

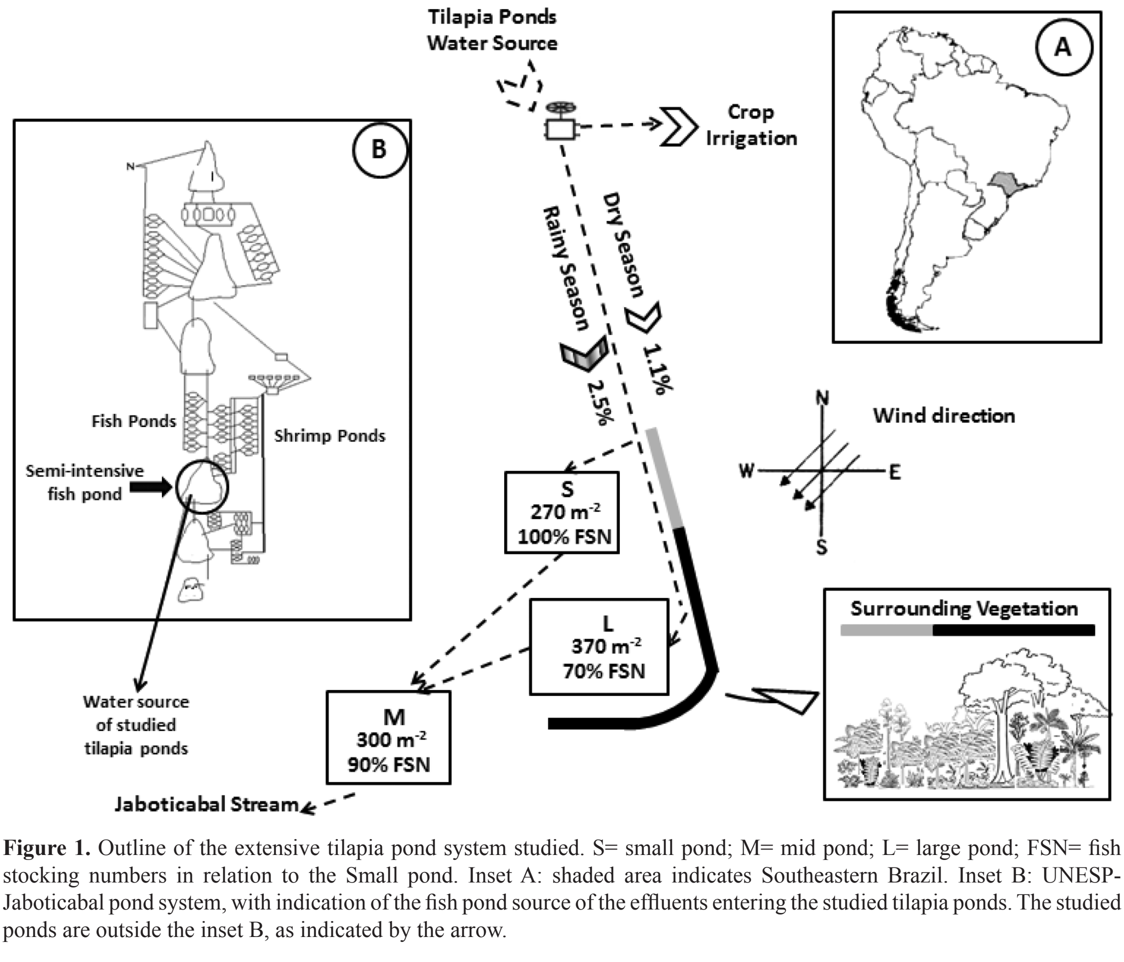

**Figure 1.** Outline of the extensive tilapia pond system studied. S= small pond; M= mid pond; L= large pond; FSN= fish stocking numbers in relation to the Small pond. Inset A: shaded area indicates Southeastern Brazil. Inset B: UNESP-Jaboticabal pond system, with indication of the fish pond source of the effluents entering the studied tilapia ponds. The studied ponds are outside the inset B, as indicated by the arrow.

understanding pond response to seasonal water flow and management operations has important implications for a successful fish production. The present research studied ecological processes developing during the rainy and the dry seasons in extensive flow-through earthen tilapia ponds fertilized with lettuce wastes.

## 2. Material and Methods

### 2.1. Pond management

This research was carried out in the fish farm at the Agricultural Technical School (CTA) of the College of Agricultural and Veterinary Sciences, University of São Paulo State (UNESP), Jaboticabal, SP (Figure 1). The three ponds studied were 1 m deep with surface area of 270 $m^2$, 300 $m^2$ and 370 $m^2$, named S (small), M (mid) and L (large), respectively. Their source water were run-off during the rainy period and the effluents of a semi-intensive fish pond (5,670 $m^2$) with high nutrient and organic matter concentrations (Lachi and Sipaúba-Tavares, 2008). The 5,670 $m^2$ pond fed a distribution box, which in turn fed the three other ponds. Two of those ponds were used for this study (ponds S and L). Water from ponds S and L served as input for pond M.

During the rainy season (October to March) water exchange between upstream and downstream ponds was continuous, with a water renewal of 2.5% of the pond volume per day, estimated from pond discharge. During the dry season (April to September) water exchange among the ponds was carried out only one to three times per month, and water renewal was reduced to the equivalent of 1.1% of the pond volume per day.

Before starting the study ponds were dried and vegetation grew on the bottom substrate. The vegetation covered at most 25% of the bottoms. The vegetal cover served as a starting source of organic fertilization for the pond sediment. After two weeks ponds were stocked with 15 g Nile tilapia at a density of 27-37 fish per pond (about 1 fish per 10 $m^2$). Lettuce inputs were performed weekly at early morning, at a rate of 15 kg fresh weight/pond/week, resulting in the equivalent input of 1.3, 1.2 and 0.9 kg/ha of nitrogen, and 0.2, 0.18 and 0.15 kg/ha of phosphorus, respectively in the S, M and L ponds. No commercial feeds were supplied to the fish, and no aeration or water mixing devices were used. After 330 days, the three ponds were partially drained and tilapia batch-weighed.

### 2.2. Environmental sampling

Water, plankton and sediment variables were sampled monthly during one year at early morning, in the middle between two lettuce inputs. *In situ* measurements included temperature, pH and dissolved oxygen (DO) with a Horiba U-10 multi-electrode, and water transparency with a Secchi disk. Water for chemical and plankton analyses were collected at about 1.5 m from the pond bank opposite to the water input pipe, at 0.5 m depth using a 5 L van Dorn sampler. For zooplankton, 10 L of water were filtered through a net of 58 µm pore, concentrated to 50 mL with 4% final concentration formalin. For phytoplankton, 5L of water were filtered through a net of 25 µm pore, concentrated to 50 mL and preserved in a lugol-iodine solution and stored in 300 mL dark bottles. Sediment samples were collected from the upper 5 cm of the pond bottom using a cylindrical PVC tube. Sediment samples were air dried, crushed and sifted through a 0.25 mm pore sieve.

### 2.3. Laboratory analyses

Concentrations in water of phosphate (PO4-P, ascorbic acid method), nitrite (NO2-N, using sulfanilamide and naftil colorimetric reagents) and nitrate ($NO_3$-N, using Cadmium to reduce it to nitrite) were determined according to Golterman et al. (1978). Ammonia levels were determined spectrophotometrically with the phenolic acid method (Koroleff, 1976) and total alkalinity ($CaCO_3$) with the phenolphthalein method (Mackereth et al., 1978). For chlorophyll-a, the water was filtered through Whatman glass fiber filters (0.45 µm pore), extracted during 24 hours with wormed ethanol 90%, and read in a colorimeter following Nusch (1980).

Concentrations in sediment of total nitrogen (Nsed, digestion with sulphuric acid), total phosphorus (Psed, calcination with sodium carbonate) and organic matter (OMsed, calcination method) were determined following Andersen (1976).

Cladocera and Copepoda were identified in a reticulated acrylic chamber under stereomicroscope (40x augmentation). Rotifera and phytoplanktonic algal units (isolated cells, colonies, filaments) were identified and counted in Sedgewick-Rafter chamber under a Leitz microscope (100x augmentation) after 30 min sedimentation. Plankton concentrations were expressed as number of organisms per water volume following APHA (1992).

### 2.4. Statistical analyses

Ecological processes that account for the main variability of the measured variables were identified through factor analysis (Milstein, 1993), which explains the relationships among measured variables by a limited number of new variables denominated factors (Johnson, 1998). The factors were extracted from principal components calculated from the correlation matrix among measured variables, and those with Eigenvalues >1 were selected for interpretation (Basilevsky, 1994). The coefficients defining the factors with absolute values indicating over 25% of the variability of a variable (coefficients >0.5) were used for factor interpretation (Dillon and Goldstein, 1984). The effects of season (rainy or dry) and pond (3 ponds) on each measured variable and factor were tested with ANOVA, and the significant main effects (season and pond) were tested with the Duncan multiple comparison test of means at a significance level of 0.05 (Zar, 1999). Plankton counts were log transformed to normalize before performing the ANOVAs, but their means were presented untransformed in tables.

## 3. Results

### 3.1. Fish growth

In spite of being an extensive system in which no tilapia mortality was observed, differences in fish growth among ponds were noted (Table 1). The M pond was stocked 10% less than the S pond, resulting in 12% higher growth rate and harvesting weight in the former and the same harvesting biomass and yield. The L pond was stocked 30% less than the S pond, resulting in only 17% higher growth rate and harvesting weight and 15% reduced harvesting biomass and yield in relation to the S pond.

### 3.2. Water, sediment and plankton

The model applied accounted for almost all (over 90%) the variability of BOD and water renovation rate, over 50% of the variability of most water quality and sediment parameters, around 30% of zooplankton groups variability, around 20% for total phytoplankton and green algae, but was not significant for phosphate and cyanobacterial groups (Table 2). Season was the main source of variability of most water quality and sediment parameters, while pond was the main variability source of total phytoplankton and all zooplankton groups.

### 3.3. Factor analysis of the environmental parameters

Four factors accounted for 77% of the overall data variability (Table 3). The first factor (Factor1) accounted for 45% of the data variability. It shows two groups of variables negatively correlated between them, indicating photosynthesis in the water column and its relation with nutrients in the sediment. Photosynthesis is enhanced by water transparency, and through this process oxygen is released into the water, pH increased, chlorophyll synthesized, and green algae develop (variables with positive coefficients), while nitrogenous nutrients are absorbed and hence decrease in the water (variables with negative coefficients). The increase of green algae is accompanied by the increase of their cladoceran grazers (positive coefficient). The amounts of nitrogenous nutrients in the water are positively correlated to the amount of nutrients in the sediments. Electroconductivity also participate in this factor, with positive coefficient. The ANOVA model applied accounted for 64% of Factor1 variability, of which two thirds were due to season (significantly higher in the dry season) and one third to pond (significantly higher in the L pond).

The second factor (Factor2) accounted for a further 15% of the data variability. It shows positive correlation among zooplankton, BOD, TSS and to a lower extent with organic matter in sediments. Thus, the factor indicates heterotrophic activity: the higher the amount of zooplankton and of particles with their attached bacteria, the higher the biological oxygen demand for respiration and decomposition respectively. The ANOVA model applied accounted for 69% of Factor2 variability, of which 40% were due to season (significantly higher in the rainy season) and 60% to pond (significantly increasing with pond size).

The third factor (Factor3) accounted for a further 9% of the overall data variability. It shows copepods and their nauplia positively correlated between them (positive coefficients), and both negatively correlated with organic loading (BOD and TSS, negative coefficients). Thus, over the variability related to biological activity of total zooplankton (Factor2), the copepod population developed more under organic loading. This variability was related to pond (significantly higher in the M pond) and not to season.

The fourth factor (Factor4) accounted for a further 7% of the overall data variability. It shows phosphate negatively correlated with Cyanobacteria. Thus, this factor indicates phosphate absorption as important process for the development of Cyanobacteria, leading to this nutrient decrease from the water. This factor was not related either to season or to pond.

## 4. Discussion

The three ponds studied are rather similar, but still present some differences in size (10-20% in area), surrounding vegetation (wind protection, amount of shadow, leaves,

**Table 1.** Stocking density, harvest and fish growth data in the three extensive tilapia ponds fertilized with lettuce waste during rearing period of 330 days.

| Parameter | Units | Small pond | Mid pond | Large pond |
|---|---|---|---|---|
| Pond area | $m^2$ | 270 | 300 | 370 |
| Total lettuce input | dry kg/ha | 1500 | 1300 | 1000 |
| Tilapia stocking | | | | |
| Density | fish/ha | 1296 | 1167 | 946 |
| Weight | Gram | 15 | 15 | 15 |
| Biomass | kg/ha | 19.4 | 17.5 | 14.2 |
| Tilapia harvesting | | | | |
| Weight | Gram | 490 | 540 | 570 |
| Biomass | kg/ha | 635 | 630 | 539 |
| Yield | kg/ha | 615 | 612 | 525 |
| Growth rate | gram/day | 1.4 | 1.6 | 1.7 |
| Survival | % | 100 | 100 | 100 |

**Table 2.** Results of ANOVA and Duncan mean multiple comparisons of the variables measured. Plankton counts were log transformed to normalize before performing the ANOVA, means presented untransformed in the table.

| | **Temp** | **EC** | **pH** | **DO** | **Chl-a** | **Secchi** | **TAN-N** | **NO$_2$-N** |
|---|---|---|---|---|---|---|---|---|
| | **(°C)** | **(µS/cm)** | | **(mg/L)** | **(µg/L)** | **(cm)** | **(mg/L)** | **(mg/L)** |
| **ANOVA Models** | | | | | | | | |
| Sign. | *** | *** | *** | *** | *** | *** | ** | ** |
| $r^2$ | 0.65 | 0.65 | 0.52 | 0.53 | 0.75 | 0.59 | 0.31 | 0.38 |
| **Sources of variance** | | | | | | | | |
| | Sign<br>%SS | Sign<br>%SS | Sign<br>%SS | Sign<br>%SS | Sign<br>%SS | Sign<br>%SS | Sign<br>%SS | Sign<br>%SS |
| Season | *** 100 | * 12 | *** 74 | *** 64 | *** 86 | *** 80 | * 36 | *** 82 |
| Pond | ns 0 | *** 88 | * 26 | ** 36 | ** 14 | * 20 | * 64 | ns 18 |
| **Mean multiple comparisons by season** | | | | | | | | |
| Rainy | 24.7 a_ | 95 _b | 7.1 _b | 5.1 _b | 360 _b | 26 _b | 0.121 a_ | 0.016 a_ |
| Dry | 23.0 _b | 100 a_ | 7.4 a_ | 5.5 a_ | 392 a_ | 32 a_ | 0.120 _b | 0.013 _b |
| **Mean multiple comparisons by pond** | | | | | | | | |
| Small | 23.8 | 89 _b | 7.2 _b | 5.2 _b | 369 _b | 27 _b | 0.121 a_ | |
| Mid | 23.8 | 104 a_ | 7.2 _b | 5.3 _b | 372 _b | 40 a_ | 0.120 ab | |
| Large | 23.8 | 101 a_ | 7.4 a_ | 5.5 a_ | 384 a_ | 31 a_ | 0.119 _b | |

| | **NO$_3$-N** | **PO$_4$-P** | **TTS** | **BOD** | **Nsed** | **Psed** | **OMsed** | |
|---|---|---|---|---|---|---|---|---|
| | **(mg/L)** | **(mg/L)** | **(mg/L)** | **(mg/L)** | **(mg/g)** | **(mg/g)** | **(%/g)** | |
| **ANOVA Models** | | | | | | | | |
| Sign. | *** | ns | *** | *** | *** | ** | *** | *** |
| $r^2$ | 0.68 | 0.17 | 0.76 | 0.92 | 0.65 | 0.39 | 0.68 | 0.96 |
| **Sources of variance** | | | | | | | | |
| | Sign<br>%SS | Sign<br>%SS | Sign<br>%SS | Sign<br>%SS | Sign<br>%SS | Sign<br>%SS | Sign<br>%SS | Sign<br>%SS |
| Season | *** 88 | * 63 | *** 41 | *** 14 | *** 55 | *** 79 | *** 98 | *** 100 |
| Pond | * 12 | ns 37 | *** 59 | *** 86 | *** 45 | ns 21 | ns 2 | ns 0 |
| **Mean multiple comparisons by season** | | | | | | | | |
| Rainy | 0.174 a_ | 0.167 | 27 a_ | 6.2 a_ | 108.1 a_ | 108.3 a_ | 7.8 a_ | 2.5 a_ |
| Dry | 0.132 _b | 0.178 | 17 _b | 5.1 _b | 105.7 _b | 107.9 _b | 5.4 _b | 1.1 _b |
| **Mean multiple comparisons by pond** | | | | | | | | |
| Small | 0.162 a_ | 0.173 | 18.7 _b | 4.7 _b | 108 a__ | 108.3 | 6.7 | 1.9 a_ |
| Mid | 0.154 ab | 0.167 | 17.3 _b | 4.7 _b | 107 _b_ | 108.2 | 6.7 | 1.7 ab |
| Large | 0.143 _b | 0.178 | 30.3 a_ | 7.7 a_ | 106 __c | 108 | 6.3 | 1.8 _b |

| | **Cyano bacteria** | **Chloro phytes** | **Phyto plankton** | **Cladocera** | **Copepoda** | **Nauplia** | **Rotifera** | **Zoo plankton** |
|---|---|---|---|---|---|---|---|---|
| | **(ind/mL)** | **(ind/mL)** | **(ind/mL)** | **(ind/L)** | **(ind/L)** | **(ind/L)** | **(ind/L)** | **(ind/L)** |
| **ANOVA Models** | | | | | | | | |
| Sign. | ns | * | * | ** | ** | * | * | ** |
| $r^2$ | 0.02 | 0.22 | 0.22 | 0.29 | 0.38 | 0.29 | 0.28 | 0.38 |
| **Sources of variance** | | | | | | | | |
| | Sign<br>%SS | Sign<br>%SS | Sign<br>%SS | Sign<br>%SS | Sign<br>%SS | Sign<br>%SS | Sign<br>%SS | Sign<br>%SS |
| Season | ns | ns 14 | ns 14 | ns 3 | ns 2 | ns 0 | ns 4 | ns 0 |
| Pond | ns | * 86 | * 85 | ** 97 | *** 98 | ** 100 | ** 96 | *** 100 |
| **Mean multiple comparisons by season** | | | | | | | | |
| Rainy | 8.2 | 94 | 107 | 79 | 17 | 69 | 37 | 203 |
| Dry | 11 | 118 | 135 | 54 | 18 | 29 | 29 | 130 |
| **Mean multiple comparisons by pond** | | | | | | | | |
| Small | 8.6 | 67 _b | 80 _b | 20 _b | 3 _b | 9 _b | 22 _b | 53 _b |
| Mid | 9.8 | 84 _b | 100 _b | 54 a_ | 34 a_ | 939 a_ | 23 _b | 204 a_ |
| Large | 10.3 | 166 a_ | 184 a_ | 126 a_ | 17 a_ | 449 a_ | 56 a_ | 242 a_ |

$r^2$= coefficient of determination. Significance levels: *= 0.05. **= 0.01. ***= 0.001. ns=not significant. %SS= percentage of total sum of squares. Same letters in each column indicate no significant differences at the 0.05 level. a>b>…. n=36 observations.

**Table 3.** Results of factor analysis, ANOVA and Duncan mean multiple comparisons of the extracted factors.

| **Factor:** | **Factor1** | **Factor2** | **Factor3** | **Factor4** |
|---|---|---|---|---|
| **Water parameters** | | | | |
| Electro Conductivity | **0.59** | 0.28 | 0.21 | 0.02 |
| pH | **0.86** | –0.20 | –0.11 | –0.01 |
| Dissolved Oxygen | **0.86** | 0.07 | –0.05 | 0.27 |
| Chlorophyll-a | **0.90** | –0.25 | –0.01 | 0.11 |
| Transparency | **0.76** | –0.21 | 0.23 | 0.28 |
| TAN-N | **–0.85** | –0.19 | 0.17 | 0.23 |
| $NO_2$-N | **–0.88** | 0.17 | 0.19 | 0.26 |
| $NO_3$-N | **–0.81** | 0.29 | –0.08 | –0.19 |
| $PO_4$-P | 0.37 | –0.20 | 0.05 | **0.69** |
| TSS | –0.10 | **0.59** | **–0.60** | 0.27 |
| BOD | 0.11 | **0.74** | **–0.46** | 0.27 |
| **Sediment parameters** | | | | |
| Nsed | **–0.92** | –0.05 | –0.02 | 0.04 |
| Psed | **–0.91** | 0.03 | 0.09 | 0.08 |
| OMsed | **–0.87** | **0.42** | 0.01 | 0.05 |
| **Plankton parameters** | | | | |
| Cyanobacteria | 0.40 | 0.29 | –0.06 | **–0.60** |
| Chlorophytes | **0.65** | 0.36 | –0.18 | –0.32 |
| Cladocera | **0.59** | **0.53** | 0.18 | 0.07 |
| Copepoda | 0.02 | **0.59** | **0.72** | 0.07 |
| Nauplia | 0.29 | **0.56** | **0.67** | –0.05 |
| Rotifera | 0.17 | **0.63** | –0.21 | 0.17 |
| Explained variance (%) | 45% | 15% | 9% | 7% |
| **Factor Interpretation** | **Photosynthesis, Nutrients in sediments** | **Heterotrophic activity** | **Copepods *vs.* Organic loading** | **Cyanobacteria development** |
| **ANOVA Models** | | | | |
| Significance | *** | *** | *** | ns |
| $r^2$ | 0.64 | 0.69 | 0.54 | 0.15 |
| **Sources of variance** | Sign %SS | Sign %SS | Sign %SS | Sign %SS |
| Season | *** 68 | *** 39 | ns 6 | ns |
| Pond | *** 32 | *** 61 | *** 94 | ns |
| **Mean multiple comparisons by season** | | | | |
| Rain season | _b | a_ | a | a |
| Dry season | a_ | _b | a | a |
| **Mean multiple comparisons by pond** | | | | |
| Small | _b | __c | _b | a |
| Mid | _b | _b_ | a_ | a |
| Large | a_ | a__ | _b | a |

Underlined and bold factor coefficients were used for interpretation. $r^2$= coefficient of determination. Significance levels: *= 0.05. **= 0.01. ***= 0.001. ns= not significant. %SS= percentage of total sums of squares. Same letters in each column indicate no significant differences at the 0.05 level. a>b>…. n=36 observations.

flowers and fruits falling into the ponds, and nutrient input by runoff), fish stocking density and water circulation patterns (location of input pipe and draining system). These differences were enough to introduce variability among ponds. Season was the main variability source of most water quality and sediment parameters, while pond was the main variability source of total phytoplankton and all zooplankton groups.

### *4.1. Dynamics of the ponds in the rainy and dry seasons*

Based on the factor analysis results, Figure 2 presents a conceptual model of the dynamics of the ponds in the rainy and dry seasons. During the warm rainy season (October-March, summer) all the output water from the source pond is available to the studied ponds. The large amounts of water entering the studied ponds bring in allochthonous

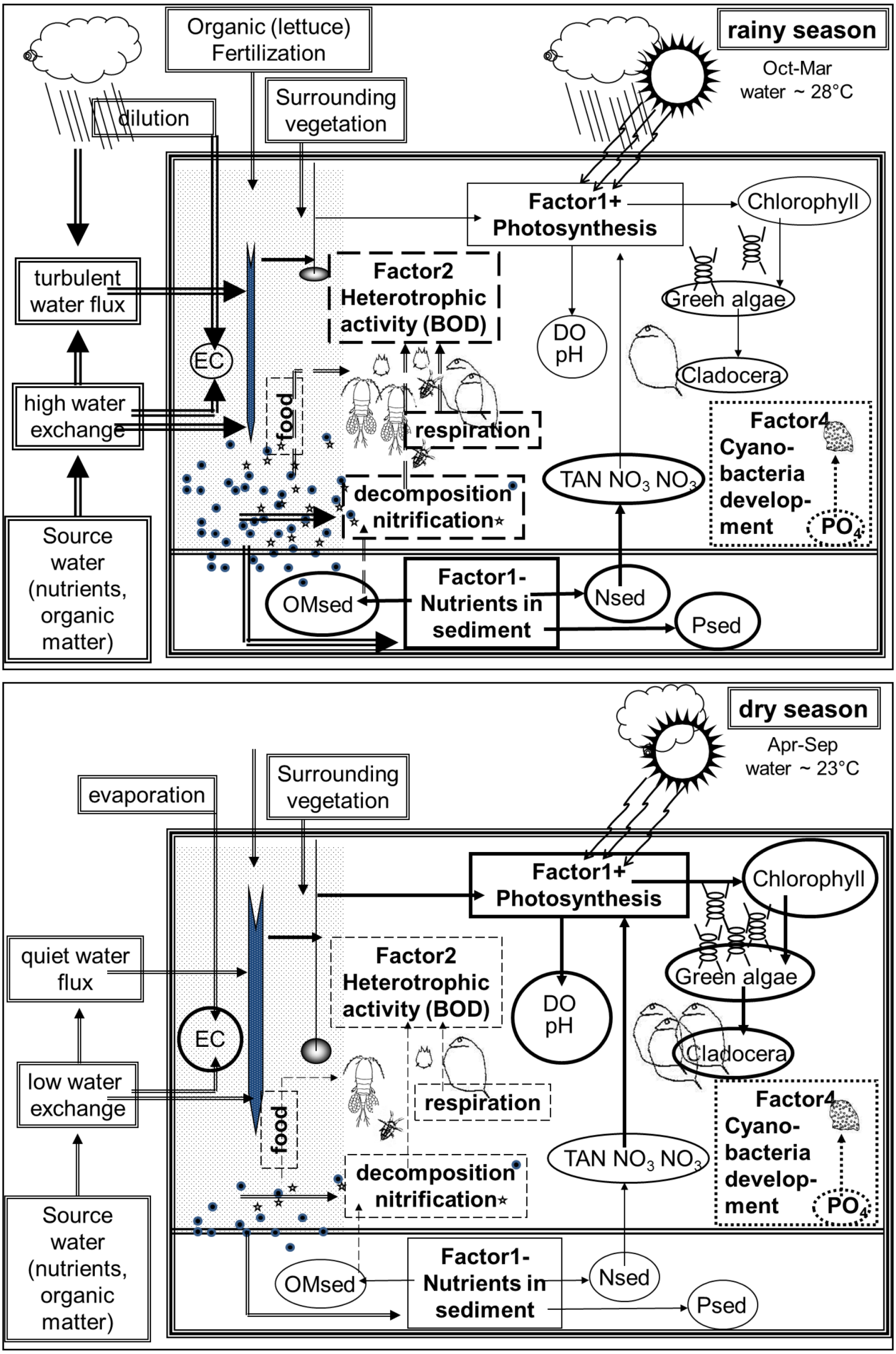

**Figure 2.** Conceptual representation of the functioning of the ponds during the rainy (upper section) and dry (lower section) periods, based on the factor analysis results. Number of organisms, and size and line thickness of boxes and arrows represent importance of effects. Boxes and arrows with single full line correspond to Factor1, broken lines to Factor2, dotted lines to Factor4, and double lines correspond to background information.

materials, and lead to higher water exchange rates. Rain and large amounts of water inflows lead to low salinities and decreased electroconductivity (Milstein et al., 2005), as well as higher turbulence, which maintains materials suspended in the water column (reduced Secchi disc visibility). Turbidity and cloudiness (rain) reduce photosynthesis (Factor1+), hence dissolved oxygen, pH, chlorophyll-a, algal populations, and cladoceran grazers. Additionally, nitrogenous nutrients are left unabsorbed, remaining in the water column. The large amounts of allochthonous materials coming with the source water, from organic fertilization, from the surrounding vegetation, and their mixing in the aerobic water column favor heterotrophic activity (Factor2): decomposition and nitrification by bacteria and respiration by the large zooplankton population that used the large amounts of suspended particles as their main food source. Additionally, decomposition of the large amounts of allochthonous materials enriched sediments (Factor1-), promoting a strong transfer of nitrogenous nutrients into the water column, enhanced by turbulent mixing (Lefebvre et al., 2001; Das et al., 2005).

During the cool dry season (April-September) water availability from the source pond is reduced, hence decreasing water inflow for the studied ponds. With rather stagnant waters (low water exchange) and high evaporation (dry weather) salts concentrate and electroconductivity increases. With rather stagnant waters and low inflows, the decreased amounts of allochthonous materials coming with the source water and from organic fertilization and surrounding vegetation settle on the pond bottom. This adds organic matter and nutrients to the sediments and keep water clear (high Secchi disc visibility). Under these conditions Factor1+ increases: clear water favors photosynthesis, which increases pH and dissolved oxygen in the water column and absorbs nitrogenous nutrients. Photosynthesis leads to increased chlorophyll-a, mainly due to the development of Chlorophytes that were the dominant planktonic algae in our samples. In turn, the green algae allow the development of their cladoceran grazers. On the pond bottom settling organic materials provide substrate for decomposing and nitrifying bacteria, which enrich the sediment providing nitrogenous nutrients to the water column (Factor1–), are a food source for zooplankton, and together with the respiration of the zooplankton increase the biological oxygen demand in the pond (Factor2). However, in quiet waters these effects are limited because the settling materials lead to anoxic conditions in the sediment, whereas nitrification and the dominant decomposition processes in the ponds require aerobic conditions (Jiménez-Montealegre et al., 2002; Avnimelech and Ritvo, 2003).

Unlike processes related to nitrogen cycling (Factor1 and Factor2), processes related to phosphorus cycling showed no differences between seasons. The development of the second most abundant phytoplanktonic group, the Cyanobacteria, required uptake of phosphate from the water column (Factor4). Many Cyanobacteria, notably *Microcystis* that was the dominant genus present in the samples, are able to fix atmospheric nitrogen. In the absence of nitrogen limitation, phosphorus becomes the limiting nutrient for their growth (Dokulil and Teubner, 2000; Moutin et al., 2002; Tanaka et al., 2004). Throughout the studied period phosphate concentration in the water column varied within a narrow range without significant differences between seasons or ponds, which was also observed with the Cyanobacteria (Table 2).

### *4.2. Variability among ponds*

The Factor1 variability among ponds is related to surrounding vegetation, which provided wind protection decreasing turbulence. Decreased turbulence allowed (a) clearer water, hence more photosynthesis (Factor1+) (Diana et al., 1991), and (b) particle sedimentation that accumulated on the pond bottom creating anaerobic conditions, which reduced sediment enrichment through aerobic decomposition and nitrification (Factor1–) (Avnimelech, 1999). The dominant winds in the area were from the NE (Figure 1) and higher Factor1 values occurred in the L pond, which is surrounded to its north and east sides by trees and shrubs. The S pond had high vegetation only in its northern side and the M pond was not surrounded by vegetation, resulting in lower Factor1 values for both ponds.

The Factor2 pond size effect was related to tilapia grazing on crustacean zooplankton (Drenner et al., 1984; Beveridge and Baird, 2000). This is because fish stocking numbers were the same in the three ponds, so that fish density per unit area decreased with increasing pond size. As a result, the S pond had higher fish density that exerted more grazing pressure on the zooplankton and suspended particles with bacteria (lower Factor2) than the M pond, and fish grazing pressure was stronger in the M than in the L pond.

The Factor3 variability among ponds was related to differences in organic loading entering the ponds with the source water and from the surrounding vegetation. As indicated above, the source water for the studied pond system had high organic loading entering the L and S ponds. These are also the ponds surrounded by vegetation; hence also received large amounts of falling leaves (in the dry season), flowers and fruits (in the rainy season). Organic loading (Factor3–) increased oxygen consumption (mainly at night), which should have affected copepod population size (Factor3+, low amounts of nauplia and copepods). The M pond receives water from the L and S ponds after some sedimentation and decomposition occurred there. Hence, the latter act as particle traps (Wahab et al., 2003) reducing organic loading entering into the M pond, which also lacks falling debris from surrounding vegetation. Under these conditions a larger copepod population developed (higher Factor3 values in pond M).

### *4.3. Tilapia performance and culture conditions*

The experiment was conducted in a rearing system that follows the usual protocols of small-scale producers in Southeastern Brazil. Thus, we were only able to follow their routine practices and we were unable to include a control pond without lettuce in the study design. So far, data regarding lettuce inputs in fishponds are inexistent. Hence, we don't have precise elements to quantify lettuce effects either on the environment or on tilapia performance. On the other hand, nutrient inputs from lettuce waste were proportional to tilapia stocking density, fish survival was 100% in all ponds, and no signs of negative effects attributable to lettuce inputs was observed (water quality within favorable range), suggesting that lettuce addition did not negatively affect tilapia growth. Together with this, there are indications that letuce addition acted as organic fertilizer for the plankton food web (as shown by Factor1) and supplemented food for tilapias (microorganisms adhered to lettuce debris of high protein level as shown by Factor2). Thus, the ecological processes described in the present research had direct effects on tilapia growth.

As seasonal conditions changed, there was a good synchrony between feeding preferences of juvenile and adult tilapias with plankton development in the ponds. Juvenile tilapias are visual predators feeding primarily on large preys such as cladocerans, while adult tilapias are filter-feeders grazing more on phytoplankton (Drenner et al., 1984; Beveridge and Baird, 2000). Juveniles were stocked in the rainy season when heterotrophic activity was higher (largely due to zooplankton density, Factor2) whereas adults were exposed to the higher photosynthesis (including Chlorophytes density, Factor1) of the dry season. Therefore, different ecological processes in the ponds and increasing stocking densities, both affected tilapia food availability and intraspecific competition, resulting in differences in tilapia growth among ponds.

In the L pond, decreased turbulence due to wind protection from the surrounding vegetation favored photosynthesis and cladoceran development (Factor1). Additionally, the lower tilapia density lowered predation pressure on zooplankton (Factor2). Together, these factors increased food availability per individual tilapia, leading to less intraspecific competition, hence higher growth rate. In the M pond the lack of surrounding vegetation did not enhance photosynthesis and cladoceran development (Factor1), whereas the reduced amounts of organic loading entering the pond enhanced copepod development (Factor3), which are fast swimming zooplankters more difficult to pray upon than cladocerans (Moss, 1980). These differences in natural food availability for tilapia, together with a higher predation pressure on zooplankton (Factor2) due to the 30% higher tilapia density in pond M, resulted in increased intraspecific competition leading to a 5% lower tilapia harvesting weight and length than in the larger pond. In the S pond, without an increase in food availability (low Factor1 and Factor3), a 10% increase in tilapia density exerted a higher predation pressure on zooplankton (Factor2), resulting in increased intraspecific competition and a 10% reduction in tilapia harvesting weight and length.

## 5. Concluding Remarks

In the extensive flow-through earthen pond system studied the main differences in pond ecology were due to different inflow dynamics during the rainy and the dry seasons. The main differences among ponds were due to effects of wind protection by surrounding vegetation, organic loading entering the ponds, tilapia density and its grazing pressure on crustacean zooplankton. Differences in tilapia growth among ponds were related to stocking density and ecological processes in the ponds, both affecting food availability and intraspecific competition.

Lettuce waste inputs used in this research did not produce negative effects, thus this practice may be considered a disposal alternative for lettuce growers and a cheap source of organic fertilizer and food for tilapia, as well as it might foster low-cost tilapia culture by resource-limited farmers.

Although the system studied was extensive, tilapia density had an impact on pond ecology and tilapia performance. With increased fish density in semi-intensive ponds those effects will probably be stronger.

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