# Effects of nutrients increase on the copepod community of a reservoir using cages

Efeitos do incremento de nutrientes sobre a comunidade de copépodes em um reservatório com tanques-rede

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Abstract: Aim: This study investigated changes in copepod abundance and the influence of environmental variables in a reservoir with fish farming using cages, on temporal and spatial scales. We hypothesised that the copepod abundance will increase when influenced by changes in environmental variables due the increase of nutrients originating from fish farming. Methods: A 120-day sampling of copepods and environmental variables was carried out in a subtropical reservoir of the Paraná River basin (Rosana reservoir), upstream and downstream of three sets of cages with different fish stocking densities. A Principal Component Analysis was conducted to characterise sampling periods and points through environmental variables. The differences between copepod abundance according to sampling periods and points were tested by ANOVA. Results: We observed higher maximum values for most nutrient concentrations and dissolved oxygen in the final stage of the experiment and in the location of cages installation. The copepod abundance increased sharply during the first days of the experiment and then decreased during the following periods with a tendency towards returning to the initial conditions at the final stage of the experiment. A significant difference in copepod abundance between the location of cages installation and downstream was showed. In addition, turbidity, chlorophyll-a, and nitrate significantly predicted copepod abundance. Conclusion: The results suggested that the changes in copepod abundance over time are influenced by environmental variables, evidenced by the increase in nutrient concentration after the cage installation, related to the increase in the practice of fish farming. The environmental variables related to system productivity were linked to the availability of food resources. Thus, our hypothesis that copepod abundance is increased due the alterations in environmental variables caused by the increase in the practice of fish farming using cages was corroborated.

Keywords: zooplankton; copepoda abundance; environmental variables; fish farming.

Resumo: Objetivo: Este estudo investigou a variação na abundância dos copépodes e a influência das variáveis ambientais nessa variação em uma área de reservatório com atividade de piscicultura em tanques-rede, em escala temporal e espacial. Nossa hipótese foi que a abundância de copépodes aumentará influenciada pelas alterações nas variáveis ambientais causadas pelo aumento dos nutrientes provenientes deste tipo de atividade. Métodos: As amostragens foram realizadas em um reservatório subtropical, na bacia do rio Paraná (reservatório de Rosana), a montante e a jusante de três conjuntos de tanques-rede com diferentes densidades de estocagem de peixes, durante 120 dias. Uma Análise de Componentes Principais foi realizada para caracterizar os períodos e pontos de amostragem por meio de variáveis ambientais. As diferenças entre a abundância de copépodes de acordo com os períodos e pontos de amostragem foram testadas pela ANOVA. Resultados: Foram observados maiores valores máximos para a maioria das concentrações de nutrientes e de oxigênio dissolvido na fase final do experimento e no local de instalação dos tanques-rede. A abundância de copépodes aumentou bruscamente nos primeiros dias do experimento e, posteriormente, diminuiu nos tempos seguintes. Foi observada uma diferença significativa na abundância de copépodes entre o local de instalação dos tanques e a jusante. As variáveis ambientais turbidez, clorofila-a e nitrato foram significativas para predizer a abundância de copépodes. **Conclusões**: Os resultados sugerem que as alterações na abundância dos copépodes ao longo do tempo são influenciadas pelas alterações das variáveis ambientais, evidenciadas pelo aumento das concentrações de nutrientes, após a instalação dos tanques, relacionadas ao incremento da atividade de piscicultura. As variáveis ambientais relacionadas à produtividade do sistema foram relacionadas à disponibilidade de recursos alimentares. Dessa forma, a hipótese de que a abundância dos copépodes aumenta devido às alterações das variáveis ambientais, causadas pelo incremento da atividade de piscicultura em tanques-rede, foi corroborada.

**Palavras-chave:** zooplâncton; abundância de copepoda; variáveis ambientais; piscicultura.

#### 1. Introduction

Fish farming using cages is a major emerging aquaculture modality in Brazilian reservoirs (Ayroza et al., 2006); the technique is relatively cheap and simple compared to traditional fish farming using earthen ponds for enabling the use of a large variety of aquatic environments such as reservoirs, and discards the costs of pond construction (Rotta & Queiroz, 2003). It is a very intensive type of farming that employs a considerable volume of food inputs for fish production using reduced space under a high fish density, which consequently releases food waste and metabolites directly into the environment (Beveridge, 1984). These are factors that may change environmental variables, and consequently, impact aquatic communities.

Environmental variables are very important in the dynamic of aquatic communities considering that alterations in their physical and chemical factors contribute to changes in the functioning pattern of the ecosystem (Rietzler et al., 2002). In addition, those characteristics influence the physiology and behaviour of organisms, as well as the structure of communities (Dunson & Travis, 1991). In a previous study, Arrieira et al. (2015) emphasised that environmental variables are major structuring factors for communities of testate amoeba in aquatic environments; therefore, the assessment of the environmental characteristics of water (physical, chemical and biological variables) may be a good predictor to analyse the variability and structure of communities in freshwater environments (Neiff, 1996).

Copepods are zooplanktonic organisms that provide good responses to those environmental variations that reflect the changes of variables over time (Margalef, 1983). A few studies discuss this community response in aquatic environments by approaching copepod sensitivity to alterations in environmental factors (Sendacz, 2001; Silva & Matsumura-Tundisi, 2005; Lansac-Tôha et al., 2009); however, no studies have yet approached the response of these organisms in environments with nutrient enrichment caused by the installation of cages for fish farming. In this context, the objective of this study was to investigate the variation in copepod abundance and the influence of environmental variables in a storage tank area with fish farming using cages, on temporal and spatial scales.

Thus, our hypothesis was that the abundance of copepods would increase influenced by changes in environmental variables caused by increase of nutrients originating from fish farming.

#### 2. Material and Methods

#### 2.1. Study area

The study was carried out in a tributary (Corvo River) located on the left bank of the lake zone belonging to the Rosana reservoir (22° 36' S and 52° 52' W) close to the confluence with Paranapanema River. The Corvo River is 239 m in width and has an average depth of 7 m in the studied stretch (Figure 1).

Characteristics of the reservoir: area of 220 km<sup>2</sup>; 116 km total length; volume of 1,920 10<sup>6</sup> km<sup>3</sup> and 1,203 m<sup>3</sup>.s<sup>-1</sup> mean annual flow; 27,600 ha of flooded area, and water residence time around of 18.6 days (CESP, 1998). It has been classified as oligo-mesotrophic and, elongated, with small branches in its tributaries and banks of rooted and submerged macrophytes (Júlio Júnior et al., 1997); in addition, it is a run-of-the-river shallow reservoir with large marginal areas (Nogueira et al., 2002; Pagioro et al., 2005). Corvo River presents margins with grasses and initial stages of reforestation with aquatic macrophytes banks predominantly of *Egeria najas* Planchon and *Eichhornia azurea* (Swartz) Kunth.

#### 2.2. Sampling design

The 120-day experiment consisted of three sets of cages with different Nile tilapia stocking densities (*Oreochromis niloticus* Linnaeus, 1758) (50 Kg.m<sup>-3</sup> or



**Figure 1.** Study area (A) and schematic representation of the sampling points (upstream, fish cages and downstream) (B) in the Corvo River.

100 fish per m<sup>3</sup>; 75 Kg.m<sup>-3</sup> or 150 fish per m<sup>3</sup>; 100 Kg.m<sup>-3</sup> or 200 fish per m<sup>3</sup>) between April (fall) and August (winter), 2006. The cages had dimensions of  $2 \times 2 \times 1.7$  m, a volume of 6.0 m<sup>3</sup>, and 10 mm of mesh size of cages. The fish were fed three times a day and the amount of food provided was adjusted according to temporal changes in biomass and the growth of the fish in the cages ( $\leq 100$  g wet weight, 10% of extruded commercial food; 100-150 g, 5%; 160-300 g, 3%; 300-500 g, 1%). The diet was composed of two extruded commercial foods (one with 32% of crude protein for the first 30 days and another with 28% of crude protein for the next 90 days).

Sampling was carried out in triplicate before the installation of the cages (T0) at five sampling points previously established for the experiment (treatment, two distances downstream and two distances upstream). After this, we carried out another triplicate sampling at seven points consisting of the installation area of the three sets of cages (P1, P2 and P3) – two distances upstream (P4 at 100 m and P5 at 400 m), and two distances downstream (P6 at 100 m and P7 at 400 m) (Figure 1). After the first sampling (T0), the remaining sampling occurred on days 15 (T1), 30 (T2), 60 (T3), 90 (T4) and 120 (T5) of the experiment, numbering 120 samples for our study.

#### 2.3. Data sampling

The copepods were sampled on the subsurface of each sampling point during the morning using a pump motor; 200 litres of water were filtered per sample through a plankton net (68  $\mu$ m). The collected material was stored in polyethylene bottles with formaldehyde fixation (4%) and the calcium carbonate buffer properly labelled.

The physical and chemical variables of water were measured under the water surface at the limnetic zone: water temperature (°C), dissolved oxygen (mg.L<sup>-1</sup>) (YSI Model 55-12FT), electric conductivity ( $\mu$ S.cm<sup>-1</sup>) (conductivimeter Digimed), pH (pH-meter Digimed), turbidity (NTU) (digital portable turbidimeter) and total alkalinity (mEq.L<sup>-1</sup>) (Carmouze, 1994).

The samples of water were collected using a Van Dorn bottle (5 litres) and cooled for laboratory analysis of the nutrient concentrations and chlorophyll-a. These analyses established the concentrations of nitrate (NO<sub>3</sub>) ( $\mu$ g.L<sup>-1</sup>) (Giné et al., 1980), ammoniacal-N (NH<sub>3</sub>) ( $\mu$ g.L<sup>-1</sup>) (Koroleff, 1976), phosphate (PO<sub>4</sub><sup>-3-</sup>) ( $\mu$ g.L<sup>-1</sup>) (Mackereth et al., 1978), total nitrogen ( $\mu$ g.L) (Zagatto et al., 1981), total phosphorus ( $\mu$ g.L<sup>-1</sup>) (Golterman et al., 1978), and chlorophyll-a ( $\mu$ g.L<sup>-1</sup>) (Golterman et al., 1978). Copepod species were identified according to Reid (1985), Matsumura-Tundisi (1986), and Lansac-Tôha et al. (2002).

We obtained three sub-samples using Hensen-Stempel pipette (2.5 mL each) to establish the abundance of individuals, counting at least 50 individuals in a modified Sedgewick-Rafter chamber under optical microscopy (Bottrell et al., 1976). The samples with reduced number of individuals were thoroughly analysed. The final abundance was expressed in terms of individuals per cubic meter (ind.m<sup>-3</sup>).

### 2.4. Data analysis

A Principal Component Analysis (PCA) was conducted to characterize sampling periods and points through environmental variables using the statistical software PC-ORD version 5.01 (McCune & Mefford, 1999); data used were log-transformed (x+1), except pH. The criterion used to retain the axes for interpretation was the Broken-Stick, as proposed by Jackson (1993).

Non-parametric one-way tests (Kruskal–Wallis) were applied to verify significant differences between grouped points (P1, P2, P3 – cages; P4, P5 – upstream; P6, P7 – downstream) of the environmental variables in each sampling periods and each sampling location. In addition, we tested the significant differences between treatment groups (cages, upstream and downstream) using an Analysis of Variance (ANOVA) (Sokal & Rohlf, 1991).

We also performed an ANOVA to assess the differences in the copepod abundance in the sampling periods and sampling points. The assumptions of normality and homoscedasticity (homogeneity of variance) were previously verified through the Shapiro-Wilk and Levene tests, respectively. A Tukey test was also applied to compare the means between the significant differences. We considered significant results when p < 0.05.

We assessed the relationship between copepod abundance and environmental variables through a multiple regression analysis (Sokal & Rohlf, 1991). This analysis is defined by equation Y = a + b (X), where Y is the response or dependent variable (copepod abundance), a (intercept) and b (coefficient) are the constants, and X represents the explanatory or independent variables (environmental variables); data were log-transformed. The assumptions of linearity, normality, homoscedasticity and independence were previous tested. These analyses were using the Statistica software 7.0 (Statsoft, 2005),

#### 3. Results

#### 3.1. Environmental variables characterisation

The environmental variables values presented no significant differences between grouped points in each studied period. We observed higher maximum values for most nutrients concentrations (total nitrogen, ammoniacal-N, total phosphorus and phosphate), and dissolved oxygen in the final stage of the experiment (T5) (Table 1). The ANOVA results between environmental variables and sampling periods are summarised in Table 2.

The environmental variables values also presented no significant differences between grouped points in each studied location. We observed higher maximum values of total nitrogen, N-nitrate, ammoniacal-N, and total phosphorus in the location of cage installation (C), while values of chlorophyll-a concentration and phosphate were higher in the

**Table 1.** Minimum, maximum values and significance of the environmental variables recorded for each studied periods (T0 = before cage installation, T1 = 15 days, T2 = 30 days, T3 = 60 days, T4 = 90 days, and T5 = 120 days after cages installation) - Corvo River, Rosana reservoir (SP/PR).

Environmental variables	Studied periods					
Environmental variables	Т0	T1	T2	Т3	T4	Т5
Turbidity (NTU)	4.2-4.8	5.8-9.0	4.0-7.1	2.2-3.6	1.9-2.7	1.8-2.3
	<i>p</i> = 0.64	<i>p</i> = 0.96	<i>p</i> = 0.10	<i>p</i> = 0.31	<i>p</i> = 0.83	<i>p</i> = 0.17
рН	6.8-7.0	7.0-7.6	7.0-7.4	5.6-6.7	6.3-7.0	6.6-7.0
	<i>p</i> = 0.17	<i>p</i> = 0.48	<i>p</i> = 0.06	<i>p</i> = 0.47	<i>p</i> = 0.37	<i>p</i> = 0.46
Alkalinity (mEq.L <sup>-1</sup> )	236.6-259.5	277.5-303.1	214.5-429.0	212.1-330.0	255.9-358.5	279.9-354.8
	<i>p</i> = 0.46	<i>p</i> = 0.47	<i>p</i> = 0.29	<i>p</i> = 0.53	<i>p</i> = 0.96	<i>p</i> = 0.38
Conductivity (uS om-1)	35.0-38.5	39.3-43.7	52.0-56.1	43.6-66.9	41.1-52.2	43-53.3
	<i>p</i> = 0.16	<i>p</i> = 0.49	<i>p</i> = 0.12	<i>p</i> = 0.49	<i>p</i> = 0.29	<i>p</i> = 0.43
Chlorophyll-a (µg.L-1)	0.8-2.9	3.4-7.5	1.9-10.9	2.7-5.2	0.7-2.7	1.0-3.4
	<i>p</i> = 0.14	<i>p</i> = 0.48	<i>p</i> = 0.46	<i>p</i> = 0.08	<i>p</i> = 0.73	<i>p</i> = 0.38
Temperature (°C)	25.9-26.1	25.2-26.2	21.9-23.0	21.4-22.3	20.0-21.6	21.8-23.5
	<i>p</i> = 0.07	<i>p</i> = 0.06	<i>p</i> = 0.44	<i>p</i> = 0.48	<i>p</i> = 0.48	<i>p</i> = 0.46
Dissolved oxygen (mg.L-1)	5.5-6.2	6.3-7.9	6.7-8.0	7.6-8.0	7.5-7.9	7.4-9.3
	<i>p</i> = 0.18	<i>p</i> = 0.06	<i>p</i> = 0.38	<i>p</i> = 0.46	<i>p</i> = 0.48	<i>p</i> = 0.47
Total nitrogen (ug L <sup>-1</sup> )	404.5-534.7	372.3-542.8	473.9-816.5	435.3-726.0	0-552.8	450.3-985.9
	<i>p</i> = 0.51	<i>p</i> = 0.25	<i>p</i> = 0.42	<i>p</i> = 0.57	<i>p</i> = 0.57	<i>p</i> = 0.62
Nitroto (ug L-1)	175.7-195.9	159.0-221.0	217.9-252.7	236.5-259.7	168.7-233.4	189.2-250.4
Nitrate (µg.L <sup>-+</sup> )	<i>p</i> = 0.19	<i>p</i> = 0.61	<i>p</i> = 0.57	<i>p</i> = 0.54	<i>p</i> = 0.53	<i>p</i> = 0.58
Ammoniacal-N (µg.L <sup>-1</sup> )	24.5-38.7	2.6-16.3	8.8-17.0	8.0-25.5	5.6-21.6	13.3-40.1
	<i>p</i> = 0.06	<i>p</i> = 0.84	<i>p</i> = 0.88	<i>p</i> = 0.66	<i>p</i> = 0.89	<i>p</i> = 0.68
Total phoapharus (ug L-1)	12.4-16.0	13.9-19.1	15.4-23.8	12.9-17.3	12.0-16.8	10.7-27.3
iotai priospriorus (µg.L <sup>-+</sup> )	<i>p</i> = 0.26	<i>p</i> = 0.47	<i>p</i> = 0.43	<i>p</i> = 0.39	<i>p</i> = 0.51	<i>p</i> = 0.53
Phosphate (µg.L <sup>-1</sup> )	5.0-8.8	3.9-6.2	3.6-13.9	4.1-7.9	2.2-5.4	2.0-11.6
	<i>p</i> = 0.70	p= 0.37	p= 0.54	p= 0.35	p= 0.37	p= 0.26

**Table 2.** The ANOVA results of the environmental variables in the studied periods.

Environmental variables	F	р
Turbidity (NTU)	118.7	< 0.01
pН	45.2	< 0.01
Alkalinity (mEq.L <sup>-1</sup> )	34.6	< 0.01
Conductivity (µS.cm <sup>-1</sup> )	27.2	< 0.01
Chlorophyll-a (µg.L <sup>-1</sup> )	20.7	< 0.01
Temperature (°C)	307.2	< 0.01
Dissolved oxygen (mg.L <sup>-1</sup> )	23.5	< 0.01
Total nitrogen (µg.L <sup>-1</sup> )	22.0	< 0.01
Nitrate (µg.L-1)	36.6	< 0.01
Ammoniacal-N (µg.L <sup>-1</sup> )	34.2	< 0.01
Total phosphorus (µg.L <sup>-1</sup> )	28.8	< 0.01
Phosphate (µg.L <sup>-1</sup> )	6.2	0.01

downstream (D) (Table 3). The ANOVA results between environmental variables and sampling points are summarised in Table 4.

The Principal Component Analysis (PCA) described 58.1% of data variability according to sampling period and point. The first PCA axis explained 36.9% of this variation with positive influence of dissolved oxygen and total nitrogen and negative influence of pH, conductivity and temperature; the second axis provided 21.2% of

the explanation, with a positive association with temperature and turbidity and a negative association with dissolved oxygen and chlorophyll-a.

The influence of those variables on the formation of axes indicated higher environmental variability for sampling periods than for points. We verified a clear separation between periods T0, T3, T4 and T5 and periods T1 and T2, suggesting that before the installation of the cages (T0) and at the final stage of the experiment (T4 and T5), there was a higher level of influence by nitrogen and dissolved oxygen. In addition, considering the scores of axis 2, we verified higher separation between sampling periods T0 and T1 (before the installation of the cages, and at day 15) with variation presenting negative association with values of pH, conductivity, temperature, turbidity and total phosphorus (Figure 2).

# 3.2. Composition and abundance of community of copepod

The copepods composition were represented by nine species, including seven Cyclopidae (Cyclopoida) species: *Mesocyclops aspericornis* (Daday, 1906), *Mesocyclops longisetus longisetus* 

	Sampling points			
Environmental variables	U	С	D	
	1.8-8.0	1.9-9.0	1.8-7.8	
Turbiality (NTU)	<i>p</i> = 0.34	Sampling points           C $1.9-9.0$ $p = 0.08$ $6.3-7.0$ $p = 0.07$ $240.3-423.8$ $p = 0.07$ $240.3-423.8$ $p = 0.07$ $36.0-54.0$ $p = 0.08$ $0.8-5.2$ $p = 0.13$ $20.0-25.9$ $p = 0.29$ $6.0-7.8$ $p = 0.79$ $415.1-985.9$ $p = 0.20$ $183.5-259.7$ $p = 0.10$ $10.2-40.1$ $p = 0.46$ $11.5-27.3$ $p = 0.75$ $2.2-11.6$ $p = 0.27$	<i>p</i> = 0.26	
	6.5-7.6	6.3-7.0	5.6-7.3	
рн	p= 0.40	Sampling points           C $3.0$ $1.9$ - $9.0$ $34$ $p$ = $0.08$ $7.6$ $6.3$ - $7.0$ $40$ $p$ = $0.07$ $422.0$ $240.3$ - $423.8$ $2^{\circ}$ $15$ $p$ = $0.07$ $36.9$ $36.0$ - $54.0$ $36.0$ $29$ $p$ = $0.08$ $7.5$ $7.5$ $0.8$ - $5.2$ $24$ $24$ $p$ = $0.13$ $26.2$ $20.0$ - $25.9$ $25$ $24$ $p$ = $0.79$ $79$ $790.6$ $415.1$ - $985.9$ $212$ $p$ = $0.79$ $790.6$ $415.1$ - $985.9$ $12$ $p$ = $0.10$ $250.4$ $183.5$ - $259.7$ $16$ $22$ $p$ = $0.10$ $8.7$ $10.2$ - $40.1$ $13$ $p$ = $0.46$ $23.1$ $11.5$ - $27.3$ $35$ $35$ $p$ = $0.75$ $93$ $2.2$ - $11.6$ $21.6$ $22.11.6$	<i>p</i> = 0.66	
	214.5-422.0	$\begin{array}{c} p = 0.08 \\ \hline & 6.3-7.0 \\ p = 0.07 \\ \hline & 0 & 240.3-423.8 & 21 \\ \hline & p = 0.07 \\ \hline & 36.0-54.0 & 3 \\ \hline & p = 0.08 \\ \hline & 0.8-5.2 \\ \hline & p = 0.13 \\ \hline & 20.0-25.9 & 2 \\ \hline & p = 0.29 \\ \hline & 6.0-7.8 \\ \hline & p = 0.79 \\ \hline & 6 & 415.1-985.9 \\ \hline & p = 0.20 \\ \hline & 4 & 183.5-259.7 & 18 \\ \hline & p = 0.10 \\ \hline & 10.2-40.1 \\ \end{array}$	212.1-429.0	
Alkalinity (mEq.L <sup>*</sup> )	<i>p</i> = 0.15	<i>p</i> = 0.07	<i>p</i> = 0.14	
	35.0-66.9	Sampling points           C           1.9-9.0         1 $p$ = 0.08 $p$ 6.3-7.0         5 $p$ = 0.07 $p$ 0         240.3-423.8         212 $p$ = 0.07 $p$ 36.0-54.0         36 $p$ = 0.08 $p$ 0.8-5.2         0. $p$ = 0.13 $p$ 20.0-25.9         20 $p$ = 0.29 $p$ 6.0-7.8         5 $p$ = 0.79 $p$ 6         415.1-985.9         0 $p$ = 0.20 $p$ 10.2-40.1         5 $p$ = 0.46 $p$ 11.5-27.3         10 $p$ = 0.75 $p$ 2.2-11.6         2. $p$ = 0.27 $p$	36.7-56.1	
Conductivity (µS.cm <sup>-+</sup> )	p= 0.29	<i>p</i> = 0.08	<i>p</i> = 0.11	
	1.3-7.5         0.8-5.2           p= 0.24         p= 0.13	0.6-10.9		
Chiorophyli-a (µg.L <sup>-+</sup> )	p= 0.24	<i>p</i> = 0.13	<i>p</i> = 0.33	
Tomporature (°C)	21.3-26.2	20.0-25.9	20.5-26.0	
Temperature (C)	<i>p</i> = 0.65	21.3-26.2     20.0-25.9       p= 0.65     p= 0.29		
Discolved evygen (mg L 1)	5.5-9.3	Sampling pointsJCD $\cdot 8.0$ $1.9-9.0$ $1.8-7.8$ $\cdot 0.34$ $p = 0.08$ $p = 0.26$ $\cdot 7.6$ $6.3-7.0$ $5.6-7.3$ $\cdot 0.40$ $p = 0.07$ $p = 0.66$ $\cdot 422.0$ $240.3-423.8$ $212.1-429.0$ $\cdot 0.15$ $p = 0.07$ $p = 0.14$ $\cdot 66.9$ $36.0-54.0$ $36.7-56.1$ $\cdot 0.29$ $p = 0.08$ $p = 0.11$ $\cdot 7.5$ $0.8-5.2$ $0.6-10.9$ $\cdot 0.24$ $p = 0.13$ $p = 0.33$ $\cdot 26.2$ $20.0-25.9$ $20.5-26.0$ $\cdot 0.65$ $p = 0.29$ $p = 0.79$ $\cdot 9.3$ $6.0-7.8$ $5.6-8.0$ $\cdot 0.67$ $p = 0.79$ $p = 0.28$ $\cdot 790.6$ $415.1-985.9$ $0-826.4$ $\cdot 0.12$ $p = 0.20$ $p = 0.20$ $\cdot 250.4$ $183.5-259.7$ $184.2-252.7$ $\cdot 0.22$ $p = 0.10$ $p = 0.30$ $\cdot 38.7$ $10.2-40.1$ $5.6-27.6$ $\cdot 0.13$ $p = 0.75$ $p = 0.68$ $\cdot 9.3$ $2.2-11.6$ $2.0-13.9$ $\cdot 0.47$ $p = 0.27$ $p = 0.58$	5.6-8.0	
Dissolved oxygen (mg.L *)	<i>p</i> = 0.67		<i>p</i> = 0.28	
	326.3-790.6	Sampling points           C $1.9-9.0$ 1 $p = 0.08$ $p$ : $6.3-7.0$ 5 $p = 0.07$ $p$ : $240.3-423.8$ 212 $p = 0.07$ $p$ : $240.3-423.8$ 212 $p = 0.07$ $p$ : $36.0-54.0$ 36 $p = 0.08$ $p$ : $0.8-5.2$ $0.$ $p = 0.13$ $p$ : $20.0-25.9$ $20$ $p = 0.29$ $p$ : $6.0-7.8$ $5$ $p = 0.79$ $p$ : $415.1-985.9$ $0$ $p = 0.20$ $p$ : $10.2-40.1$ $5$ $p = 0.10$ $p$ : $10.2-40.1$ $5$ $p = 0.46$ $p$ : $11.5-27.3$ $10$ $p = 0.75$ $p$ : $2.2-11.6$ $2$	0-826.4	
lotal hitrogen (µg.∟*)	<i>p</i> = 0.12		<i>p</i> = 0.20	
Nitrata (	159.0-250.4	UC-8.0 $1.9-9.0$ $1.8$ $0.34$ $p = 0.08$ $p =$ -7.6 $6.3-7.0$ $5.6$ $0.40$ $p = 0.07$ $p =$ -422.0 $240.3-423.8$ $212.1$ $0.15$ $p = 0.07$ $p =$ -66.9 $36.0-54.0$ $36.7$ $0.29$ $p = 0.08$ $p =$ -7.5 $0.8-5.2$ $0.6$ $0.24$ $p = 0.13$ $p =$ -26.2 $20.0-25.9$ $20.5$ $0.65$ $p = 0.29$ $p =$ -9.3 $6.0-7.8$ $5.6$ $0.67$ $p = 0.79$ $p =$ -790.6 $415.1-985.9$ $0.8$ $0.12$ $p = 0.20$ $p =$ -250.4 $183.5-259.7$ $184.2$ $0.22$ $p = 0.10$ $p =$ -23.1 $11.5-27.3$ $10.7$ $0.35$ $p = 0.75$ $p =$ -9.3 $2.2-11.6$ $2.0-27$ $0.47$ $p = 0.27$ $p =$	184.2-252.7	
	p= 0.22		<i>p</i> = 0.30	
Ammonicool N (ug L-1)	2.6-38.7	10.2-40.1	5.6-27.6	
Ammoniacai-N (µg.L ·)	<i>p</i> = 0.13	<i>p</i> = 0.46	<i>p</i> = 0.25	
	11.8-23.1	11.5-27.3	10.7-23.8	
iotai priospriorus (μg.L <sup>-+</sup> )	p= 0.35 p= 0.75 p=		<i>p</i> = 0.68	
December (ug L 1)	2.1-9.3	2.2-11.6	2.0-13.9	
Phosphale (µg.L.')	p= 0.47	p= 0.27	<i>p</i> = 0.58	

**Table 3.** Minimum, maximum values and significance of the environmental variables recorded for each studiedpoints (U = upstream; C = cage installation location; D = downstream) - Corvo River, Rosana reservoir (SP/PR).

**Table 4.** The ANOVA results of the environmentalvariables in the studied points.

Environmental variables	F	р
Turbidity (NTU)	3.4	0.03
рН	3.2	0.04
Alkalinity (mEq.L <sup>-1</sup> )	3.3	0.04
Conductivity (µS.cm <sup>-1</sup> )	4.0	0.02
Chlorophyll-a (µg.L⁻¹)	4.9	< 0.01
Temperature (°C)	3.2	0.04
Dissolved oxygen (mg.L <sup>-1</sup> )	3.7	0.03
Total nitrogen (µg.L <sup>-1</sup> )	2.3	0.01
Nitrate (µg.L-1)	3.1	0.03
Ammoniacal-N (µg.L-1)	7.0	< 0.01
Total phosphorus (µg.L <sup>-1</sup> )	3.7	0.03
Phosphate (µg.L <sup>-1</sup> )	4.1	0.02

(Thiébaud, 1912), Mesocyclops meridianus (Kiefer, 1926), Mesocyclops ogunnus Onabamiro, 1957, Mesocyclops sp., Thermocyclops decipiens (Kiefer, 1929), and Thermocyclops minutus (Lowndes, 1934); and two Diaptomidae (Calanoida) species: Argyrodiaptomus azevedoi (Wright, 1935) and Notodiaptomus henseni (Dahl, 1894).

Notodiaptomus henseni (28.475 ind.m<sup>-3</sup>), Thermocyclops minutus (3.821 ind.m<sup>-3</sup>) and Thermocyclops decipiens (633 ind.m<sup>-3</sup>) showed the highest mean abundance in this study (Figure 3). The ANOVA results indicated significant differences in the variation of abundance according to the sampling period (F  $_{(5,114)} = 16.562$ ; p < 0.01), with a sharp increase between T0 and T2 (experiment day 30) and decrease for the following periods (T3 and T4), with a tendency to return to T5 initial conditions (final stage). The Tukey test indicated that T1 and T2 differed from each other and from the remaining periods (Figure 4A). The ANOVA results for abundance values between sampling points were also significant (F  $_{(3,114)} = 3.66$ ; p = 0.03). The Tukey test showed that the significant difference occurred between cage installation location and downstream (Figure 4B).

## 3.3. Relationship between copepod abundance and environmental variables

According to the multiple regression analysis, turbidity, chlorophyll-a, and NO<sub>3</sub> were significant for the prediction model for copepod abundance with a positive correlation to turbidity and nitrate. A negative correlation was observed to chlorophyll-a (Table 5). The following model explained 98% of data variability:  $Log_{10}$  (Cop) = 0.117 \*  $Log_{10}$  (Turb) – 0.087 \*  $Log_{10}$  (Chlor-a) + 0.956 \*  $Log_{10}$  (NO<sub>3</sub>); where: Cop = copepod abundance; Turb = turbidity;  $NO_3$  = nitrate; Chlor-a = chlorophyll-a.



**Figure 2.** Scores of the Principal Component Analysis (PCA) with dispersion of the environmental variables according to the sampling periods (A) and points (B). Environmental variables: Temp = temperature; Turb = turbidity; DO = dissolved oxygen; Cond = conductivity; TP = total phosphorus; TN = total nitrogen; chlor = chlorophyll-a.



Figure 3. Copepod abundance in the studied period. Species: A. a.: Argyrodiaptomus azevedoi; N. h.: Notodiaptomus henseni; M. sp: Mesocyclops sp.; M. a.: Mesocyclops aspericornis; M. l. l.: Mesocyclops longisetus longisetus; M. m.: Mesocyclops meridianus; M. o.: Mesocyclops ogunnus; T. d.: Thermocyclops decipiens; T. m.: Thermocyclops minutus.



**Figure 4.** Copepod abundance in the different sampling periods (A) and points (B). Symbol = mean abundance values (ind.m<sup>-3</sup>); vertical bars = standard error.

**Table 5.** Summary of the multiple regression model between copepod abundance (response variable) and values of turbidity, chlorophyll-a, and nitrate (explanatory variables); t corresponds to t test of parameters significance; p is the probability to find t higher than the obtained.

	Parameters (β)	Partial correlation coefficient	t	р
Turbidity	0.117	0.742	2.145	0.03
Chlorophyll-a	-0.087	-0.418	-2.3	0.02
Nitrate	0.956	0.408	18.37	< 0.01

#### 4. Discussion

The practice of fish farming using cages caused evident alterations on the environmental characteristics and, consequently, on copepod abundance, considering the sampled periods and points. These variations can be regarded as factors related to major anthropic activities influencing the community of microcrustaceans (Illyová & Pastuchova, 2012).

In fish farming using cages, uneaten food, faeces, mucus and soluble wastes (phosphorus and nitrogen compounds) are directly dispersed into the water, resulting in an eutrophication process (Demir et al., 2001). This impact influences the copepod abundance by direct forces, altering the physical and chemical characteristics of the water column in the reservoirs, or by indirect forces, starting with impacts caused by food resource availability. For example, this increase in the quantity of nutrients after establishment of the cage causes an increase in phytoplankton biomass, resulting in a high biomass of invertebrates (Guo & Li, 2003), which can used by copepods as a food resource.

The higher environmental variable concentration values and significant differences were verified in the final stage of the experiment (T5), and cages and downstream. These results could be caused by the introduction of artificial foods in the cages that directly affected the environment with increase of nutrient concentration. The alterations in the environment may also influence the dynamics of the copepods community in response to this environmental stress (Demir et al., 2001; Matsumura-Tundisi & Tundisi, 2003).

The results of the regression analysis suggest that the increase in system productivity related especially to suspended solids (turbidity), chlorophyll-a, and nitrate could influenced changes in the community abundance according to sampling periods and points. These environmental variables related to system productivity are linked to the availability of food resources (Auer et al., 2004) and directly affected copepod abundance. Copepods can feed on bacteria, small food particles and algae (Matsumura-Tundisi & Tundisi, 2005), that show similar spatial variation and higher abundance on productivity environments (Dias et al., 2011).

According to the PCA results and alterations in community abundance, as evidenced by the ANOVA results, there is a high primary production and changes in the system productivity, revealing alterations in community abundance, influencing its stability (Sommer et al., 1986; Tilman et al., 2006). In addition, the increase in productivity increases rates of individual growth and reproduction of organisms, and thereafter, the rapid increase in population abundance (Gliwicz, 2002; Dias et al., 2012). Those results were revealed through the negative association with phosphorus and the positive with nitrogen – major environmental variables related to productivity.

A rapid increase in copepod abundance until day 30, after the installation of the cages, and the posterior decrease after this abundance peak, could be attributed to a higher initial increase and after decreases in the phytoplankton and heterotrophic flagellate abundance (Borges et al., 2010). These results are in agreement with Dias et al. (2011), who observed a peak of abundance in the first days after the installation of the cage due to the contribution of the young and adult stage copepods. The authors also demonstrated that a positive correlation between copepod and phytoplankton could explain the high copepod abundance observed.

A possible explanation for the high abundance of Notodiaptomus henseni (Calanoida) is that these species are not influenced by alterations in environmental variables (Perbiche-Neves et al., 2013). The higher abundance of Cyclopoida species represented by Thermocyclops minutus and T. decipiens can be explained considering that these species are commonly abundant and are found sharing habitats in oligo-mesotrophic environments, such as the conditions of the studied reservoir (Silva & Matsumura-Tundisi, 2005; Landa et al., 2007; Nogueira et al., 2008; Perbiche-Neves et al., 2013). In addition, Thermocyclops decipiens has been employed as a water bio-indicator with high nutrient concentration (Rocha et al., 2002; Landa et al., 2007; Nogueira et al., 2008).

Environmental variables, such as turbidity, chlorophyll-a, and nitrate, were regarded as regulatory strengths, acting on copepod abundance for influencing both its temporal and spatial distribution, which states that the practice of fish farming can affect environmental conditions. The lower amplitude of food resources, which could have intensified the interspecific competition, could infer the decrease in copepod abundance in the final studied periods. The abundance of these organisms depends on the support capability of the environment and the ability of species to use the resources and explore different niches (Matsumura-Tundisi et al., 1990).

Therefore, the hypothesis that the community of copepod abundance increases due to alterations in environmental variables caused by the increase in the practice of fish farming using cages was corroborated.

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### References

- ARRIEIRA, R.L., ALVES, G.M., SCHWIND, L.T.F. and LANSAC-TÔHA, F.A. Local factors affecting the testate Amoeba community (Protozoa: Arcellinida; Euglyphida) in a neotropical floodplain. *Journal of Limnology*, 2015, 73(AoP). http://dx.doi.org/10.4081/ jlimnol.2015.1078.
- AUER, B., ELZER, U. and ARNDT, H. Comparison of pelagic food webs in lakes along a trophic gradient and with seasonal aspects: influence of resource and predation. *Journal of Plankton Research*, 2004, 26(6), 697-709. http://dx.doi.org/10.1093/plankt/fbh058.
- AYROZA, D.M.M.R., FURLANETO, F.P.B. and AYROZA, L.M.S. Regularização dos projetos de tanques-rede em águas públicas continentais de domínio da união no estado de São Paulo. *Boletim Técnico do Instituto de Pesca*, 2006, 36, 1-32.
- BEVERIDGE, M.C.M. Cage and pen fish farming: carrying capacity models and environmental impact. Rome: FAO, 1984. 131 p. FAO Fisheries Technical Paper, 255.
- BORGES, P.A.F., TRAIN, S., DIAS, J.D. and BONECKER, C.C. Effects of fish farming on plankton structure in a Brazilian tropical reservoir. *Hydrobiologia*, 2010, 649(1), 279-291. http://dx.doi. org/10.1007/s10750-010-0271-2.
- BOTTRELL, H.H., DUNCAN, A., GLIWICZ, Z.M., GRYIEK, E., HERZIG, A., HILLBRICHT-ILKOWSKA, A., KURASAWA, H., LARSSON, P. and WEGLENSKA, T. A review of some problems in zooplankton production studies. *Norwegian Journal of Zoology*, 1976, 24, 419-456.
- CARMOUZE, J.P. O metabolismo dos ecossistemas aquáticos: fundamentos teóricos, métodos de estudo e analises química. São Paulo: Edgard Blücher/FAPESP, 1994. 253 p.
- COMPANHIA ENERGÉTICA DE SÃO PAULO - CESP. Conservação e manejo nos reservatórios: limnologia, ictiologia e pesca. São Paulo: CESP, 1998. 166 p. Série Divulgação e Informação.
- DEMIR, N., KIRKAGAC, M.U., PULATSU, S. and BEKCAN, S. Influence of trout cage on water quality, plankton and benthos in an anatolian Dam Lake. *Israeli Journal of Aquaculture*, 2001, 53(3-4), 115-127.
- DIAS, J.D., SIMÓES, N.R. and BONECKER, C.C. Zooplankton community resilience and aquatic environmental stability on aquaculture practices: a study using net cages. *Brazilian Journal of Biology*, 2012., 72(1), 1-11. PMid:22437379.
- DIAS, J.D., TAKAHASHI, E.M., SANTANA, N.F. and BONECKER, C.C. Impact of fish cage-culture on the community structure of zooplankton in a tropical reservoir. *Iheringia-Série Zoologia*, 2011, 101(1-2), 75-84. http://dx.doi.org/10.1590/S0073-47212011000100011.
- DUNSON, W.A. and TRAVIS, J. The role of abiotic factors in community organization. *American*

*Naturalist*, 1991, 138(5), 1067-1091. http://dx.doi. org/10.1086/285270.

- GINÉ, M.F., BERGAMIN, F., ZAGATTO, E.A.G. and REIS, B.F. Simultaneous determination of nitrate and nitrite by flow injection analysis. *Acta Analytica Chimica*, 1980, 114, 191-197. http://dx.doi. org/10.1016/S0003-2670(01)84290-2.
- GLIWICZ, Z.M. On the different nature of top-down and bottom-up effects in pelagic food webs. *Freshwater Biology*, 2002, 47(12), 2296-2312. http://dx.doi. org/10.1046/j.1365-2427.2002.00990.x.
- GOLTERMAN, H.L., CLYMO, R.S. and OHMSTAD, M.A.M. *Methods for physical and chemical analyses of freshwaters*. Oxford: Blackwell Scientific Publication, 1978. 213 p.
- GUO, L. and LI, Z. Effects of nitrogen and phosphorus from fish cage-culture on the communities of a shallow lake in middle Yangtze River basin of China. *Aquaculture (Amsterdam, Netherlands)*, 2003, 226(1-4), 201-212. http://dx.doi.org/10.1016/S0044-8486(03)00478-2.
- ILLYOVÁ, M. and PASTUCHOVÁ, Z. The zooplankton communities of small water reservoirs with different trophic conditions in two catchments in western Slovakia. *Limnologica*, 2012, 42(4), 271-281. http:// dx.doi.org/10.1016/j.limno.2012.08.004.
- JACKSON, D.A. Stopping rules in principal component analysis: a comparison of heuristical and statistical approaches. *Ecology*, 1993, 74(8), 2204-2214. http:// dx.doi.org/10.2307/1939574.
- JÚLIO JÚNIOR, H.F., BONECKER, C.C. and AGOSTINHO, A.A. Reservatório de Segredo e sua inserção na bacia do rio Iguaçu. In A.A. AGOSTINHO and L.C. GOMES, orgs. *Reservatório de segredo: bases ecológicas para o manejo*. Maringá: Eduem, 1997. 387 p.
- KOROLEFF, K.J.H. Determination of ammonia. In E. GRASSHOFF and E. KREMLING, orgs. *Methods of seawater analysis.* New York: Verlag Chemie Weinheim, 1976. 317 p.
- LANDA, G.G., BARBOSA, F.A.R., RIETZLER, A.C. and MAIA-BARBOSA, P.M. *Thermocyclops decipiens* (Kiefer, 1929) (Copepoda, Cyclopoida) as indicator of water quality in the State of Minas Gerais. *Brazilian Archives of Biology and Technology*, 2007, 50(4), 695-705. http://dx.doi.org/10.1590/S1516-89132007000400015.
- LANSAC-TÔHA, F.A., BONECKER, C.C., VELHO, L.F., SIMÓES, N.R., DIAS, J.D., ALVES, G.M. and TAKAHASHI, E.M. Biodiversity of zooplankton communities in the Upper Paraná River floodplain: interannual variation from long-term studies. *Brazilian Journal of Biology*, 2009, 69(2,Suppl. Suppl), 539-549. http://dx.doi.org/10.1590/S1519-69842009000300009. PMid:19738961.
- LANSAC-TÔHA, F.A., VELHO, L.F.M., HIGUTI, J. and TAKAHASHI, E.M. Cyclopidae (Crustacea, Copepoda) from the upper Paraná river floodplain,

Brazil. *Brazilian Journal of Biology*, 2002, 62(1), 125-133. http://dx.doi.org/10.1590/S1519-69842002000100015. PMid:12185912.

- MACKERETH, F.J.H., HERON, J. and TALLING, J.F. *Water analysis: some revised methods for limnologists.* Kendal: Titus Wilson & Son Ltd, 1978. 117 p.
- MARGALEF, R. *Limnologia*. Barcelona: Omega, 1983. 1010 p.
- MATSUMURA-TUNDISI, T. and TUNDISI, J.G. Calanoida (Copepoda) species composition changes in the reservoirs of São Paulo State (Brazil in the last twenty years). *Hydrobiologia*, 2003, 504(1-3), 215-222. http://dx.doi.org/10.1023/ B:HYDR.0000008521.43711.35.
- MATSUMURA-TUNDISI, T. and TUNDISI, J.G. Plankton richness in eutrophic reservoir (Barra Bonita reservoir, SP, Brazil). *Hydrobiologia*, 2005, 542(1), 367-378. http://dx.doi.org/10.1007/s10750-004-9461-0.
- MATSUMURA-TUNDISI, T. Latitudinal distribution of Calanoida in freshwater aquatic systems of Brazil. *Brazilian Journal of Biology*, 1986, 46(3), 527-553.
- MATSUMURA-TUNDISI, T., LEITÁO, S.N., AGUENA, L.S. and MIYAHARA, J. Eutrofização da represa de Barra Bonita: Estrutura e organização da comunidade de Rotifera. *Brazilian Journal of Biology*, 1990, 50, 923-935.
- MCCUNE, B. and MEFFORD, M. *Multivariate Analysis* of *Ecological Data*. Version 4.01. Oregon: MJM Software Design, 1999.
- NEIFF, J.J. Large rivers of South America: toward the new approach. *Verhandlungen des Internationalen Verein Limnologie*, 1996, 26(1), 167-180.
- NOGUEIRA, M.G., JORCIN, A., VIANNA, S.C. and BRITTO, Y.C. Uma avaliação dos processos de eutrofização nos reservatórios em cascata do rio Paranapanema (SP/PR), Brasil. In A.F. CIRELLI and G.C. MARQUISÁ, orgs. *El agua em Iberoamérica: de la limnologia a la gestión em Sudamérica*. Santiago do Chile: CYTED XVII/CETA, 2002. p. 91-106.
- NOGUEIRA, M.G., REIS-OLIVEIRA, P.C. and BRITTO, Y.T. Zooplankton assemblages (Copepoda and Cladocera) in a cascade of reservoirs of a large tropical river (SE-Brazil). *Limnetica*, 2008, 27, 151-170.
- PAGIORO, T.A., THOMAZ, S.M. and ROBERTO, M.C. Caracterização limnológica abiótica dos reservatórios. In L. RODRIGUES, S.M. THOMAZ, A.A. AGOSTINHO and L.C. GOMES, orgs. *Biocenoses em reservatórios: padrões espaciais e temporais.* São Carlos: Rima, 2005. 333 p.
- PERBICHE-NEVES, G., FILETO, C., LAÇO-PORTINHO, J., TROGUER, A. and SERAFIM-JÚNIOR, J. Relations among planktonic rotifers, cyclopoid copepods, and water quality in two Brazilian reservoirs. *Latin American Journal of Aquatic Research*, 2013, 41(1), 138-149. http://dx.doi.org/10.3856/ vol41-issue1-fulltext-11.

- REID, J.W. Chave de identificação e lista de referências bibliográficas para as espécies continentais sulamericanas de vida livre da ordem Cyclopoida (Crustacea, Copepoda). *Boletim de Zoologia*, 1985, 9, 17-143.
- RIETZLER, A.C., MATSUMURA-TUNDISI, T. and TUNDISI, J.G. Life cycle, feeding and adaptive strategy implications on the co-occurrence of Argyrodiaptomus furcatus and Notodiaptomus iheringi in Lobo-Broa reservoir (SP, Brazil). *Brazilian Journal of Biology*, 2002, 62(1), 93-105. http:// dx.doi.org/10.1590/S1519-69842002000100012. PMid:12185928.
- ROCHA, M.I.A., BRANCO, C.W.C., SAMPAIO, G.F., GÔMARA, G.A. and DE FILIPPO, R. Spatial and temporal variation of limnological features, *Microcystis aeruginosa* and zooplankton in an eutrophic reservoir (Funil Reservoir, Rio de Janeiro). *Acta Limnologica Brasiliensia*, 2002, 14(3), 73-86.
- ROTTA, M.A. and QUEIROZ, J.F. Boas práticas de manejo (BPMs) para produção de peixes em tanques-rede. Corumbá: Embrapa Pantanal, 2003. 27 p.
- SENDACZ, S. Planktonic Copepoda of the Upper Paraná River Floodplain lakes (São Paulo and Mato Grosso do Sul, Brazil). *Hydrobiologia*, 2001, 453/454(1), 367-374. http://dx.doi.org/10.1023/A:1013138017196.
- SILVA, W.M. and MATSUMURA-TUNDISI, T. Taxonomy, ecology, and geographical distribution of the species of the genus Thermocyclops Kiefer, 1927 (Copepoda, Cyclopoida) in São Paulo State, Brazil, with description of a new species. *Brazilian Journal of Biology*, 2005, 65(3), 521-531. http:// dx.doi.org/10.1590/S1519-69842005000300018. PMid:16341431.
- SOKAL, R.R. and ROHLF, F.J. Biometry: the principles and pratice of statistics in biological research. New York: WH Freeman and Company, 1991. 887 p.
- SOMMER, U., GLIWICZ, Z.M., LAMPERT, W. and DUNCAN, A. The PEG-model of seasonal succession of plankton in fresh waters. *Archiv für Hydrobiologie*, 1986, 106(4), 433-471.
- STATSOFT. Statistica for Windows (data analysis software system), version 7.1. Tulsa: Statsoft Inc, 2005.
- TILMAN, D., REICH, P.B. and KNOPS, J.M.H. Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature*, 2006, 441(7093), 629-632. http://dx.doi.org/10.1038/nature04742. PMid:16738658.
- ZAGATTO, E.A.G., JACINTO, A.O., REIS, B.F., KRUG, F.G., BERGAMIN, F.H., PESSENDA L.C.R., MORTATTI, J. and GINÉ, M.F. Manual de análises de plantas e águas empregando sistema de injeção de fluxo. Piracicaba: Universidade de São Paulo/Centro de Energia Nuclear na Agricultura, 1981. 42 p.