Diurnal variation of zooplankton biochemical composition and biomass in plankton production tanks

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ABSTRACT. The nutritional value of zooplankton (lipid and phosphorus contents) was analyzed in outdoor, plankton-culture tanks, to monitor the effects of diurnal variation and fertilization. Total lipid contents were significantly higher (average values of about 14% DW) for the treatment fertilized during the sampling week. A significantly highher total lipid concentrations generally coincided with high biomass of *Moina* sp. (Cladocera) and *Thermocyclops* sp. nauplii (Copepoda), which is probably related to zooplankton filtration rates. The values tended to be higher during the afternoon and the night. Phosphorus contents in the zooplanktonic population varied from 0.15 to 0.91% DW, with significant differences among the tanks. The zooplankton nutritional quality was greatly influenced by fertilization, which also determined the species composition in the tanks.

Key words: zooplankton, biochemical composition, biomass, plankton tanks.

RESUMO. Variação nictemeral da composição bioquímica e biomassa do zooplâncton em tanques de cultivo de plâncton. O valor nutricional do zooplâncton (teor de lipídios e fósforo) foi analisado em tanques externos de cultivo de plâncton, sendo monitorados os efeitos da variação diurna e fertilização. O teor de lipídios foi significantemente mais elevado no tanque que sofreu fertilização na semana da coleta, com valor médio de 14% PS. Em geral, elevadas concentrações de lipídios foram observadas quando a biomassa de *Moina sp.* (Cladocera) e de náuplios de *Thermocyclops* sp. (Copepoda) também foram altas, estando relacionadas com a filtração do zooplâncton. Os valores tenderam a ser maiores durante os períodos diurno e vespertino. O teor de fósforo no zooplâncton variou entre 0,15% e 0,91% PS, com diferenças significativas entre os tanques. A qualidade nutricional do zooplâncton foi influenciada pelo regime de fertilização dos tanques, o qual também determinou a composição das espécies presentes.

Palavras-chave: zooplâncton, composição bioquímica, biomassa, tanques de cultivo de plâncton

Introduction

The production of planktonic organisms in good nutritional condition to feed fish larvae and fingerlings is a basic requirement in fish culture. In a vast majority of fish farms in Brazil, it is a common practice to add organic and chemical fertilizers into the hatchery ponds (Sá-Junior, 1994).

Although this procedure ensures a quick response in terms of algal biomass increase, both zooplankton composition and nutritional condition change abruptly, causing low fish larvae survival rates, due to the bad quality of food (Santeiro and Pinto-Coelho, 2000).

An adequate plankton biochemical composition ensures the nutritional requirements for fish larvae, especially during their initial developmental stages. The living food improvement may decrease the high fish larvae mortality rate, a common problem in fish farms (Coutteau and Sorgeloos, 1997).

Lipids are important molecules in the process of energy reserve for reproduction and growth; besides, they are necessary for the cellular membrane structure (Phleger *et al.*, 1998). The changes of total lipid in the zooplanktonic community are associated, among other factors, to the life span and the environment trophic state and also to plankton tank management (Santeiro and Pinto-Coelho, 2000).

Phosphorus content has also been utilized to evaluate zooplankton nutritional quality and as an indicator of environmental conditions to which the organisms are exposed (Gulati and Demott, 1997; Pinto-Coelho *et al*., 1997a).

Temporal variations of phosphorus contents in zooplanktonic organisms occur according to diet availability and quality. The absorption efficiency, assimilation and energy storage capacity are all intimately associated to the nutritional status of different groups of aquatic organisms (Macedo and Pinto-Coelho, 2000).

This study aimed to evaluate the variations in zooplankton nutritional status during fertilization regime in outdoor plankton tanks throughout the measurements of total lipid and phosphorus contents, during three different periods of growing fish season.

Material and methods

Study area

The study was carried out at Furnas Hydrobiology and Hatchery Station, Furnas Hydroelectric (São José da Barra, State of Minas Gerais, Brazil). The station is located on Rio Grande left bank, 2 km away from Altinopolis dam, which provides material for the FURNAS tanks. Two tanks with an area of 200 m^2 , a volume of 320 m^3 and 1.4-m deep were used for the plankton culture.

Fertilization

The fertilization was applied using chemical fertilizer (25.6 kg of simple super phosphate and 48 kg of ammonia sulfate), added only at the beginning of the experiment, and organic fertilizer, which consisted of adding 100 kg of pig manure "in natura" at intervals of approximately 7 days. However, only tank 2 (T_2) was fertilized during the sampling week, as a standard procedure adopted at this station.

Density and biomass

In both tanks the samples were collected during diurnal variation at regular 4-hour intervals during three periods: October (beginning of fish growing season), January (height of fish growing season) and March (final of fish growing season).

Zooplankton samples were obtained using a 58μm-mesh-size net and preserved in formalin/sucrose solution (4%). The zooplankton was sampled in vertical hauls of water column with 45-L filtered total. The zooplankton samples were then placed in a Sedgewick-Rafter Cell, and examined at 100-times magnification. From the measurement of each organism, an average biomass (Bi) was estimated for each phase of the zooplanktonic populations (Geller and Muller, 1985; Pinto-Coelho, 1991) and the zooplankton density (Di) was calculated according to APHA (1989).

Biochemical composition

Dry weight (DW) was determined using a Mettler high-precision balance (accuracy ± 0.1μg). The zooplankton was first freeze-dried and than transferred to small pre-weight aluminum "boats", according to Berbevoric and Pinto-Coelho (1989). The homogenized material was chemically digested. Total lipids were spectrophotometrically determined (546 nm) using the sulpho-phosphovanillin reaction, according to Meyer and Walther (1988).

Zooplankton phosphorus was analyzed by colorimetric method (Murphy and Riley, 1962). All determinations were conducted with three replicates.

Statistical analysis

Log and normal distribution Kolmogorov-Smirnov test was applied for zooplankton data. Furthermore, a parametric test with linear regression analysis (ANOVA) was applied to different tanks and sampling times, in order to test these variables with zooplankton phosphorus and total lipid contents (Two ways). Covariance analysis (ANCOVA) was used to test the biomass of each zooplanktonic population versus phosphorus and total lipid contents. For all the analyses, the 4.3 version, Statistic for Windows was used (Zar, 1996).

Results

Zooplankton

A clear quantitative change in the zooplanktonic biomass in both tanks was observed during the diurnal variations. In October, there was a dominance of *Thermocyclops* sp. nauplii and *Moina* sp.; *Brachionus calyciflorus* and *Thermocyclops* sp. adult dominated in January, and *Thermocyclops* sp. adult and *Moina* sp. in March. The average values of zooplanktonic biomass varied sharply; however, in January, T_2 showed much higher biomass than T_1, probably due to the organic fertilization. In T_2, the zooplanktonic population biomass varied from 6.14 to 187.37 μg/L in October, from 41.27 to 145.44 μg/L in January, and from 1.84 to 233.49 μg/L in March. In tank T_1, variations ranged from 15.52 to 68.12 μg/L, 6.51 to 49.64 μg/L, and 12.67 to 265.65 μg/L during the months of October, January and March, respectively (Table 1).

An inverse relationship was observed for the biomass of Rotifera *Brachionus calyciflorus* and Cyclopoida *Thermocyclops* sp. (nauplii and copepodid).

Moina sp. showed higher biomass in T_2, at 12:00 h in October, with 146.32 μg/L, and in March at 8:00 h, with 184.28 μg/L. On the other hand, the highest biomass of *Moina* sp. in T_1 was obtained in March, at 8:00 h, with only 20.68 μg/L.

A fluctuation was observed in the abundance peaks of zooplanktonic biomass. In October, the adult *Thermocyclops* sp. tended to show higher biomass during the afternoon, with maximum of 56.25 at 16:00h and 39.39 μg/L at 12:00 in T_1 and T_2, respectively. In January, the maximum in T_2 was observed at 16:00h, with 134.64 μg/L. In this period, *Thermocyclops* sp. nauplii and copepodid, despite the low biomass of nauplii and copepodid, showed low biomass in tank T_2 and were absent in T_1. In March, they appeared again; adult *Thermocyclops* sp showed the highest biomass observed during the study, 264.25 μg/L in tank 1 at 24:00h (Table 1).

Brachionus calyciflorus reached representative numbers in January. The highest biomass values were observed at 8:00 h, with 40.50 and 71.83 μg/L in T_1 and T_2, respectively. In October, this species was practically absent; it appeared at 12:00 h in tank T_1, with 0.01 μg/L and at 20:00 h in T_2, 0.13 μg/L. It was also observed that the highest biomass values for *Brachionus calyciflorus* in January, at 8:00h, was related to the absence and/or low biomass level for *Thermocyclops* sp. copepodid and nauplii (Table 1).

Moina sp. was present during the whole experimental period, with higher values in tank T_2, with biomass varying from 1.65 to 146.32 μg/L; 0.01 to 13.73 μg/L, and 1.35 to 184.28 μg/L in October, January and March, respectively. In the previous month, this species had their highest biomass value at 8:00 h (Table 1).

Biochemical composition

Lipid concentration in the planktonic community oscillated from 9.14 to 16.66% DW in T_1 and from 9.93 to 16.81% DW in T_2. Although there was a visible trend for higher lipid values in the afternoons and nights, the diurnal differences were not significant. In general, lipid levels were similar in both tanks. However, in March, lipid concentration in tank T_2 was slightly lower than in T_1, varying from 9.93 to 12.94% DW, and from 12.16 to 14.86% DW, respectively (Tables 1 and 2).

Table 1. Diurnal variation of zooplankton biomass (μg/L DW), phosphorus (% DW), and lipids (% DW) in plankton tanks (T_1 and T_2) during October, January and March, where: *B*=*Brachyonus*; *T*=*Thermocyclops* sp., *A*=*Argyrodiaptomus*.

Biomass/	**October**													
Biochemical				**T_1**							**T_2**			
Zooplankton	**8:00**	**12:00**	**16:00**	**20:00**	**24:00**	**4:00**	**8:00**	**8:00**	**12:00**	**16:00**	**20:00**	**24:00**	**4:00**	**8:00**
B. calyciflorus	-	0.01	-	-	-	-	-	-	-	-	0.13	-	-	-
T. nauplii	13.12	6.80	0.03	17.79	67.94	42.52	34.72	1.01	0.89	5.88	47.24	26.46	5.86	4.18
T. copepodid	0.62	0.77	0.04	0.46	0.07	0.12	0.14	0.01	0.06	0.05	0.54	-	-	0.05
T. adult	4.26	0.81	56.25	8.27	0.10	4.87	15.63	12.88	39.39	3.18	7.28	1.64	0.28	8.46
A. furcatus	-	-	-	-	-	-	-	-	0.71	0.03	-	-	-	-
Moina sp.	0.13	7.23	0.48	2.64	0.01	-	-	48.35	146.32	3.94	113.90	-	-	1.65
Total Biomass	18.11	15.64	56.79	29.16	68.11	47.51	50.50	62.42	187.37	13.08	169.09	28.90	6.15	14.33
Lipids	11.74	12.55	9.14	10.53	10.16	12.65	16.66	15.79	16.81	14.33	16.21	14.65	10.78	12.28
Phosphorus	0.91	0.66	0.80	0.85	0.62	0.67	0.78	0.79	0.68	0.87	0.56	0.75	0.46	0.68
Biomass/	**January**													
Biochemical				**T_1**							**T_2**			
Zooplankton	**8:00**	**12:00**	**16:00**	**20:00**	**24:00**	**4:00**	**8:00**	**8:00**	**12:00**	**16:00**	**20:00**	**24:00**	**4:00**	**8:00**
B. calyciflorus	5.73	25.09	25.76	19.71	19.47	17.08	40.50	71.83	36.78	10.44	26.38	47.46	14.78	44.74
T. nauplii	0.02	-	-	-	-	-	-	-	0.01	0.01	0.04	-	-	-
T. copepodid	-	-	-	-	-	-	-	-	0.13	0.01	0.28	-	-	0.65
T. adult	0.76	0.07	0.04	0.09	0.50	9.31	5.32	-	4.32	134.64	22.03	16.22	48.35	6.70
A. furcatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Moina sp.	-	0.02	0.06	0.69	6.26	1.83	3.82	-	0.03	0.34	1.49	0.74	0.01	13.73
Total Biomass	6.51	25.18	25.87	20.49	26.22	28.23	49.65	71.83	41.26	145.44	50.22	64.43	63.13	65.82
Lipids	13.80	12.88	12.99	13.28	15.19	16.47	14.64	12.33	14.07	13.69	14.83	14.83	16.80	16.33
Phosphorus	0.55	0.37	0.54	0.18	0.50	0.46	0.44	0.72	0.19	0.23	0.64	0.74	0.69	0.62
Biomass/	**March**													
Biochemical				**T_1**							**T_2**			
Zooplankton	**8:00**	**12:00**	**16:00**	**20:00**	**24:00**	**4:00**	**8:00**	**8:00**	**12:00**	**16:00**	**20:00**	**24:00**	**4:00**	**8:00**
B. calyciflorus	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T. nauplii	11.33	7.73	3.80	-	1.40	-	0.04	11.21	-	-	-	0.02	61.13	12.99
T. copepodid	0.01	-	0.01	-	-	-	-	0.06	0.01	-	-	-	0.07	0.03
T. adult	1.13	2.15	9.79	27.87	264.25	106.08	22.11	37.94	29.60	0.24	43.14	48.21	92.30	16.30
A. furcatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Moina sp.	3.59	2.39	10.88	0.60	-	0.93	20.68	184.28	1.35	1.60	60.62	51.74	4.52	15.52
Total Biomass	16.09	12.27	24.48	28.47	318.45	106.98	42.79	232.00	30.00	1.84	107.76	99.95	157.95	44.81
Lipids	13.76	14.05	12.16	13.28	14.86	13.21	14.05	11.04	12.94	12.21	11.80	9.93	12.77	12.35
Phosphorus	0.69	0.76	0.85	0.62	0.47	0.15	0.60	0.50	0.47	0.68	0.77	0.73	0.70	0.78

Phosphorus content was not significantly different with tank fertilization for *Moina* sp. and *Thermocyclops* sp., but significant differences were observed regarding the year periods. In October, the values tended to be higher between 16:00 h and 24:00 h. Phosphorus concentrations in zooplankton were below 1% DW, with the highest and lowest concentrations observed in T_1, respectively, in October at 16:00 h, with 0.91% DW, and in March at 4:00 h with 0.15% DW (Tables 1, 2, and 3).

Variance analysis for each tank, when comparing zooplanktonic population, was significant only for phosphorus contents in the zooplanktonic biomass. Lipid contents differed statistically between the tanks only for the species *Brachionus calyciflorus* (Table 3).

Table 2. Two-way ANOVA evaluation for of zooplankton lipids and phosphorus content comparing fertilization (effect 1) and time (effect 2) in the plankton tanks during experimental period.

	Tanks/ Time				
Zooplankton	Effect	Df	Ms	F	p
Phosphorus	1	1	2.55	3.91	0
	2	1	0	0	0.09
	12	1	1.81	2.78	0
Lipids	1	1	3.34	5.02	0
	2	1	0.57	0.86	0.36
	12	1	1.19	0.8	0.14

Table 3. ANOVA evaluation of lipid and phosphorus contents in zooplankton, comparing fertilization (effect 1) and time (effect 2) in the plankton tanks during experimental period.

Zooplankton	Lipids					Phosphorus				
	Effect	Df	Ms	F	p	Effect	Df	Ms	F	p
Total biomass	1	1	1.83	1.87	0.17	1	1	6.99	10.66	0.00
	2	1	1.98	2.02	0.16	2	1	2.02	3.09	0.08
	12	1	0.22	0.23	0.63	12	1	0.04	0.06	0.79
Thermocyclops sp.	1	1	2.68	2.87	0.09	1	1	11.41	14.80	0.00
nauplii	2	1	2.45	2.62	0.11	2	1	0.29	0.77	0.53
	12	1	0.00	0.00	0.96	12	1	0.00	0.77	0.96
Thermocyclops sp.	1	1	1.78	1.84	0.18	1	1	10.36	13.37	0.00
copepodid	2	1	2.20	2.27	0.13	2	1	0.24	0.31	0.57
	12	1	0.28	0.29	0.58	12	1	0.12	0.16	0.68
Thermocyclops sp.	1	1	2.10	2.30	0.13	1	1	10.95	14.04	0.00
adult	2	1	3.14	3.43	0.07	2	1	0.21	0.27	0.60
	12	1	0.57	0.62	0.43	12	1	0.06	0.08	0.77
Moina sp	1	1	1.98	2.03	0.16	1	1	10.87	13.91	0.00
	2	1	2.32	2.37	0.13	2	1	0.27	0.35	0.55
	12	1	0.25	0.26	0.60	12	1	0.11	0.14	0.70
Brachionus calyciflorus	1	1	1.96	5.00	0.03	1	1	11.01	14.39	0.00
	2	1	0.03	0.08	0.77	2	1	0.57	0.75	0.38
	12	1	0.05	0.14	0.70	12	1	0.18	0.24	0.62
Argyrodiaptomus furcatus	1	1	0.00	0.00	0.92	1	1	13.12	18.17	0.00
	2	1	1.85	3.15	0.08	2	1	0.34	0.47	0.49
	12	1	0.29	0.50	0.48	12	1	0.08	0.11	0.73

Discussion

The results showed that lipid levels in the zooplankton tended to be higher during the night. Pinto-Coelho *et al.* (1997b) also reported the highest total lipids at midnight, in a total of 25.1% DW for tanks with higher production conditions and water quality standard. According to the authors, this was probably due to the dominance of *Diaphanosoma* during the period.

Santeiro and Pinto-Coelho (2000) found, in plankton culture tanks, total lipid values for zooplankton ranging from 6-10% DW, which is considered a low nutritive value category. In this study, lipid contents of fertilized tanks were somewhat higher, ranging from 9 to 16% DW, showing that fertilization has direct impact on certain organisms and on lipid level fluctuation. This fact is probably related to the water quality, which causes the manifestation of certain species in detriment of others. Lipid fluctuations are related with changing environmental conditions and increasing filtering rates during the night (Macedo and Pinto-Coelho, 1997). In this study, the different treatments (T_1 and T_2) and the diurnal metabolic change of zooplankton activities caused a fluctuation of biomass lipid levels.

Recent theories and experimental studies suggest that the flow of one chemical element through the zooplankton is affected by the quality and quantity of available food. For example, if one element, such as nitrogen or phosphorus, is relatively scarce to fulfill zooplankton requirements, this element is then concentrated in the tissue, in order to maximize the organism`s growth (Urabe *et al.*, 1995).

In this study, the plankton food chain into the tanks was probably more associated with detritus than with algae, due to the addition of organic matter from fertilizers at short time intervals. Generally, energy transfer efficiency is much lower in such chains; however, they are an important process and have a prominent role in the nutrient cycles and dynamics of the food chain (Attayde and Ripa, 2000).

Species of Cladocera, such as *Moina* sp., store high amounts of lipid to achieve a longer life span and to increase the probability of survival with minimum food supplies (Macedo and Pinto-Coelho, 2000).

Copepoda copepodid and nauplii accumulate larger amount of lipid, according to the type of food available in the environment (Santeiro and Pinto-Coelho, 2000). Sipaúba-Tavares *et al.* (2001), verified that pacu larvae preferred Copepoda-Calanoida nauplii and *Moina micrura*, which have lipid content above 12% DW.

In this study, the highest concentration of total lipid (16.81% DW) was observed when *Moina* sp. was more abundant in the zooplanktonic community. The lowest lipid content (9.14% DW) was observed when *Thermocyclops* sp. was dominant in the zooplankton.

About the phosphorus contents in zooplankton, the levels found may be attributed to the changes in species composition. When Cladocera *Moina* sp. was dominant in the tanks, average phosphorus level was between 0.15 and 0.87% DW; for Copepode nauplii, it was between 0.46 and 0.91% DW. The lowest phosphorus values in zooplankton were detected in January, when the community was represented mainly by Rotifer.

The changes observed in the chemical composition of the zooplanktonic organisms may be related to the differences in the physiological state of these animals. The changes verified in the zooplankton nutritional status have important implications on aquaculture station management. Fertilization is necessary to obtain plankton with adequate nutritional value; however, water quality should be controlled, in order to maintain species with high nutritional values.

Additional, more detailed studies are necessary to develop a specific methodology for management of outdoor plankton tanks, aiming to improve the produced food nutritional quality, thus ensuring higher larval survival, as well as increasing the larvae feeding times when lipid concentration is higher. This methodology may consist, for example, in changing flow rates in the tanks, improving the fertilization regimen by adjusting N:P ratios offered via organic/chemical fertilizers. However, in order to achieve an objective protocol, further investigation is necessary on the factors that affect zooplankton composition, mainly about the dynamics of energy reserve accumulation.

Based on this study, it is possible to conclude that the zooplankton nutritional quality was greatly influenced by fertilization and also that water quality determined the species composition in the tanks. Zooplankton feeding behavior is directly related to the quantity of lipid stored in the animal's body and to the diurnal variation changes in zooplankton filtration rates.

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