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EFFLUENTS QUALITY DURING THE GROW-OUT PHASE OF THE AMAZON SHRIMP *Macrobrachium amazonicum*

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ABSTRACT

In recent years shrimp culture farms have been one of the most growing sectors in aquaculture. Research has been carried out in order to establish a sustainable production maintaining profit and low environmental impact. Current investigation analyzed source and effluent water produced during the final grow-out phase of the Amazon shrimp (*Macrobrachium amazonicum*). Twelve natural-bottom ponds, with continuous water flow and stock density comprising 40, 60, 80 and 100 young shrimps/m^2 were analyzed. The experiment design comprised totally randomized blocks with four treatments and three replications. Microbiological analyses for *Escherichia coli* was attempted, coupled to physical and chemical analyses for pH, temperature, total suspended solids, total nitrogen, nitrite, nitrate, biochemical oxygen demand and chemical oxygen demand of water supply and pond effluents. Results show that whereas effluent quality complied with current legal rules, there was no significant difference between supply and effluent water for the analyzed variables and between stock densities. Under the conditions investigated and the intensification of culture in the final grow-out phase up to a density of 100 young shrimps/m^2, the production of *M. amazonicum* reveals low potential for environmental impact for the variables analyzed.

KEYWORDS: effluent; environmental impact, shrimp culture, water quality.

QUALIDADE DOS EFLUENTES PRODUZIDOS NA FASE DE CRESCIMENTO DO CAMARÃO-DA-AMAZÔNIA *Macrobrachium amazonicum*

RESUMO

Nas últimas décadas, a carcinicultura de água doce foi um dos setores que mais cresceu na aquicultura. Muitas pesquisas têm sido realizadas a fim de buscar metodologias para uma produção sustentável, ou seja, lucrativa e com baixo impacto ambiental. Neste contexto, o presente estudo objetivou analisar a qualidade da água de abastecimento e do efluente gerado na fase de crescimento final do cultivo do camarão-da-amazônia (*Macrobrachium amazonicum*). Foram analisados 12 viveiros de fundo natural, com fluxo contínuo de água e densidade de estocagem de 40, 60, 80 e 100 juvenis/m^2. O delineamento experimental utilizado foi blocos inteiramente casualizados com quatro tratamentos e três repetições. Foram realizadas análises microbiológicas para *Escherichia coli* e análises físicas e químicas para pH, temperatura, sólidos totais suspensos, nitrogênio total, nitrito, nitrato, demanda bioquímica de oxigênio e demanda química de oxigênio da água de abastecimento e efluentes dos viveiros. Os resultados revelaram que a qualidade do efluente estava de acordo com a legislação vigente e que não ocorreu diferença significativa entre a água de abastecimento e efluente para as variáveis analisadas. Também não ocorreu diferença significativa entre as densidades de estocagem. Concluiu-se que a produção de *M. amazonicum* nas condições estudadas e a intensificação do cultivo na fase de crescimento final, até a densidade de 100 juvenis/m^2, para as variáveis analisadas, apresenta baixo potencial de impacto ambiental.

PALAVRAS-CHAVE: carcinicultura, efluente, impacto ambiental, qualidade de água..

INTRODUCTION

Aquaculture, greatly developed worldwide, is based on sustainability. According to Valenti[1], modern aquaculture has been founded on the concepts of profitable production, social development and preservation of the environment. The three concepts are intrinsically related and interdependent so that the activity will continue to develop.

Direct release of agricultural effluents may cause chronic bio-accumulation and eutrophization in receiving water bodies, with high phytoplankton increase and, consequently, dissolved oxygen deficit during the night and possible death of local living organisms[2].

Changes in the receiving water bodies go beyond the environmental impacts and, consequently, public health issues become relevant. Several studies show that in eutrophic environments there is a predominance of cyanobacteria which, in turn, produces toxins[3].

Increase in nitrogen levels in water ecosystems, with special reference to impacting human activities, involves not merely the eutrophization process when converted into nutrients, such as nitrate and nitrite, but may also cause metahemoglobinemia and the formation of cancerigenous nitrosamines and nitrosamides[4-6]. Due to the increase of aquacultural activity, there is also an increasing concern by environmental and health authorities because of the environmental impact produced.

Aiming at decreasing negative impacts on the aquatic environment in shrimp culture, current research has been undertaken to improve the quality and decrease the amount of effluent. Working on shrimp breeding and the environmental impact caused by such activity, Páez-Osuna[7] reported that an increasing use of antibiotics against shrimp diseases is a high concern activity, since it may possibly cause an impact in the neighboring natural ecosystem.

Fraga[8] showed that semi-intensive cultural systems of shrimp *Litopenaeus vannamei*, in the municipalities of Barra do Sul and Laguna, in the state of Santa Catarina, Brazil, increased deposits of suspended solids, chlorophyll a, phaeophytins, organic matter, feopigments, available phosphorus and total nitrogen in the waters and sediments of receiving coastal ecosystems. Benassi[9] compared the affluent and effluent water quality of a stock-maintaining pond with reproducers of the shrimp *Macrobrachium rosenbergii*. The author frequently verified increasing percentages of concentrations in total nitrogen (20% to 180%), total phosphorus (30% to 130%), turbidity values (10% to 35%) and suspended material above 50%. Increase was related to diets and shrimp excrements which caused a reduction of the effluent's water quality.

Costanzo et al.[10] undertook a study in a sea water shrimp farm in northeastern Australia and verified the water quality of a stream with effluent originating from *Penaeus merguiensis* culture. Contrastingly to values when the ponds were de-activated (1.3±0.3 μM and 1.2±0.6 $\mu g L^{-1}$, respectively), an increase in concentrations of $NH4^{+}$ (18.5±8.0 μM) and chlorophyll a (5.5±1.9 $\mu g\ L^{-1}$) was found in the water column after the ponds were stocked with young shrimps. Results show that the water quality of this particular stream was directly affected by the farm and may have been associated to algae blooms downstream the effluent release site.

Folke & Kautsky[11] suggested polyculture as an alternative, clean and sustainable technology, or rather, the culture of organisms that occupy different trophic levels and parts of the water column, with total use of several types of food, including residues. The construction of wetlands is another alternative for effluent treatments for shrimp culture and has been employed as a recirculation filter. The latter efficiently reduces phosphorus, nitrogen, ammonia, nitrate, suspended solids and biochemical oxygen demand levels[12,13].

Considering them less impacting factors, Braz Filho[14] has analyzed breeding systems in fish culture with total water recirculation, already adopted in countries such as Thailand. Water circulation meets the exigencies of the European Community whose suppliers must avoid water waste, improve the product's quality and the production system. Recirculating systems are generally associated with living organisms such as algae, bacteria, filter animals or water plants which are accountable for the removal and/or transformation of debris, making possible the reuse of water for the continuation of the culture.

The effluent's characterization and the problem's diagnosis and solutions through the employment of technologies such as aerators, proper food management, polyculture, total water circulation and employment of effluent treatment tanks with macrophytes or filter organisms, are highly important actions in order to decrease the aquaculture-caused environmental impact. However, research should be undertaken as above, especially when Brazilian species, as is the case with *Macrobrachium amazonicum*, are involved.

Generally the culture of freshwater shrimps causes less environmental impact that involves seawater. The construction of the latter farms destroys coastal swamps, a highly complex and fragile ecosystem of great importance for seawater biodiversity[15]. Owing to environmental sustainability and to the well-developed production technology of freshwater shrimps, there has been recently a fast increase in the activity[16]. The most common species in commercial cultures in several tropical and subtropical countries is the Malaysia-originating *Macrobrachium rosenbergii*[16-18].

However, the introduction of exotic species is a serious issue and has been considered by the International Union for World Conservation of Nature (IUCN) as the second most important cause in biodiversity loss. Exotic species vie for space and food with native species and may transmit viruses, bacteria and fungi to the parasites against which they lack immunity[19]. Further, exotic species with close relatives in native biota may reproduce with these specimens and eliminate the genotypes which are unique in local species. They may also bring about the loss of genetic diversity, hybrids and a lack of definition in current taxonomic limits[20, 21].

Current research is part of the Program for the Technological Development of the *M. amazonicum* culture, established at the UNESP Aquaculture Center - CAUNESP - in 1999, with the participation of ten research institutions and universities from different Brazilian regions. Due to the theme's relevance and importance and to the scantly number of research on *Macrobrachium amazonicum*, this current study evaluates source and effluent water in the species' final grow-out phase. Microbiological analyses for *Escherichia coli*, coupled to physical and chemical analyses for pH, temperature, suspended solids, BOD, COD, nitrate, nitrite and total nitrogen were undertaken. Variables analyzed at the effluents were compared with standards established by Brazilian legislation[22]. Important information that would help controlling effluents' quality for environmental conservation will be consequently obtained.

MATERIAL AND METHODS

Grow-out phase was developed by a semi-intensive system in the shrimp farm section at the UNESP Aquaculture Center in Jaboticabal, SP, Brazil. The phase started in the beginning of December 2005 through April 2006, with duration of 133 days. Twelve earth-bottom ponds, area approximately 0.01ha, were filled with freshwater. Prior to stocking, the pond's bottom was limed by 1 t/ha of hydrated lime and organic fertilization with an addition of 3 t/ha of shrimp ration.

Young shrimps weighing approximately 0.02g were stocked on the 12th December 2005. Table 1 shows stocking density of 40, 60, 80 and 100 young shrimp specimens/m^2. The experiment design comprised totally randomized blocks with four treatments and three replications, with a total of 12 ponds.

Table 1. Stocking densities, number of stocked specimens and pond area, UNESP Aquaculture Center, 2006

Pond	Stocking density	Number of stocked specimens	Area of ponds (m^2)
1	80	7120	89
2	100	8900	89
3	60	5520	92
4	40	4000	100
5	80	8080	101
6	60	6120	102
7	100	10600	106
8	40	4000	100
9	60	7440	124
10	40	4600	115
11	100	12000	120
12	80	7840	98

The animals were fed on pellet diet with a 35% crude protein, offered twice a day at 8:00 AM and 5:00 PM. Whereas daily feeding rate for each pond reached 2.5 g/m2 during the first month of culture, the daily diet in pond reached 9, 7, 5 and 3% of biomass from the second to the fourth month. The latter was weekly adjusted by 20%. Diet quantity was cut by half when dissolved oxygen level ranged between 2.5 and 3.5 mg L^{-1} in the morning. Diet was deferred when the variable's level was below 2.5 mg L^1.

Pond water came from a reservoir upstream the tanks and distributed through gravity. Water renewal rate of the ponds was between 5 and 10% per day.

Water samples from the water supply and from the effluent of each pond were collected in the morning, every fortnight, during the breeding period and in total harvest. Whereas samples for microbiological analysis were collected in pre-sterilized 250 mL flasks, samples for physical and chemical analyses were collected in 2 L bottles.

During full harvest, the pounds were emptied so that shrimps could be net-retrieved. Samples of the effluent were collected at three distinct moments: at the start of the emptying of the ponds (initial 1/3); when the pond's water was approximately half retrieved (middle 1/3); when ponds were almost empty (final 1/3).

Most Probable Number (MPN) of *Escherichia coli* was calculated according to APHA[23]. Affluent and effluent samples were diluted in sterile peptonated water 0.1%, or, rather, 10 mL of sample were added to 90 mL of the diluent, producing dilution 10^{-1}. First dilution produced the successive decimal dilutions. Culture medium (Colilert) was added to the water sample or its dilution (100 mL). After homogenization the mixture was transferred to quanti-tray wells and sealed with a specific seal. Wells were then incubated at 35 ºC during 24 h. MPN of *E. coli* was calculated after incubation by the number of fluorescent cells when UV rays were beamed on wells. Specific table was employed when sample´s MPN of E. coli/100 mL was reached.

Multi-parameter probe W-22XD Horiba determined pH values, dissolved oxygen, temperature and total solids.

Total nitrogen concentrations were obtained by semi-micro Kjedahl method through the transformation of ammonia nitrogen into ammonia (NH_3), which is fixed by boric acid and then titred with sulfuric acid (H_2SO_4) till the formation of ammonia nitrogen with the acid-base indicator[23].

Nitrate concentrations were obtained by 8039 method[24] for water and waste water, namely, cadmium-decrease method according to DR-2010 Hach on the spectrophotometer model. Nitrite concentrations were obtained by method 815324 for water and waste waters, namely, ferrous sulfate, according to DR-2000 Hach on the spectrophotometer model.

Biochemical Oxygen Demand (BOD) was obtained with AL 320 Aqualic with a solution of potassium hydroxide 45% as reagent and internal pressure sensors. Pressure changes in sealed sampled flasks were directly changed into BOD (mg L^{-1})[23]. Chemical Oxygen Demand (COD) concentrations were obtained by colorimetry through spectrophotometer and Hach digester block for COD. Methodology described in instruction books indicates acid digestion in di-potassium chromate medium and catalyzers, employing standard in the apparatus's memory[23].

Water quality variables were analyzed by Variance Analysis method (ANOVA) for totally randomized experiments. ANOVA was repeated for each variable for each cultivating data so that the effect of different effluent water density would be estimated[25].

Box-Cox transformation was applied on harvest for ANOVA so that variable which required transformation could be verified and transformation identified. Only E. coli (EC), total suspended solids (TSS), nitrate (NTRA), total nitrogen (TN) and Chemical Oxygen Demand (COD) required transformation. The latter were $\sqrt{EC + 0.01}$, $(STS)^8$, log (NTRA + 0.01), $1/(NT)^2$ and $\sqrt{\mathbf{DQO}}$ respectively[25]. After performing the required transformations, wastes of all variables complied with normality and homoscedasticity.

RESULTS AND DISCUSSION

Owing to lack of data on the species under analysis, when possible, results were compared to the species *Macrobrachium rosenbergii* and discussion on research therewith undertaken.

Final grow-out is potentially the most impacting phase within the production cycle since the water's continuous flow is used. Although continuous flow maintains the pond water's quality, it releases great quantities of effluents in the environment. Table 2 shows the results of the current research.

Table 2. Mean values, standard deviation and F Test statistics of results from microbiological analysis of fecal coliforms (PMN/100 mL of *E. coli*), pH, dissolved oxygen (DO) (mg/L), temperature (Temp) (ºC), total suspended solids (TSS) (mg/L), total nitrogen (TN) (mg/L), nitrite (mg/L), nitrate (mg/L), biochemical oxygen demand (BOD) (mg/L) and chemical oxygen demand (COD) (mg/L) of water supply and of effluents of final grow-out ponds with stocks at densities 40, 60, 80 and 100 post-larvae of *M. amazonicum*/m2, UNESP Aquaculture Center, 2006

Water analyzed	Analyzed variables					
	Supply	Effluent-D40	Effluent-D60	Effluent-D80	Effluent-D100	F_treatment*
E. coli	84.27(±86.90)	47.27(±130.06)	30.16(±54.09)	7.14(±253.20)7	53.16(±144.24)	1.03NS
pH	7.71(±0.27)	7.59(±0.50)	7.59(±0.43)	7.52(±0.36)	7.61(±0.49)	0.36NS
DO	8.60(±2.95)	8.27(±2.76)	8.43(±2.91)	8.43(±2.90)	8.38(±2.87)	0.02NS
Temp.	27.13(±1.35)	27.63(±1.63)	27.41(±1.39)	27.47(±1.44)	27.52(±1.31)	0.20NS
TSS	0.06(±0.01)	0.07(±0.02)	0.06(±0.01)	0.06(±0.01)	0.06(±0.02)	0.05NS
TN	0.44(±0.31)	0.45(±0.23)	0.48(±0.30)	0.48(±0.26)	0.46(±0.20)	0.12NS
Nitrite	0.01(±0.01)	0.01(±0.01)	0.01(±0.01)	0.01(±0.01)	0.01(±0.01)	0.29NS
Nitrate	0.92(±0.40)	0.74(±0.61)	0.46±(0.45)	0.59(±0.42)	0.68(±0.49)	1.46NS
BOD	8.44(±2.77)	11.75(±9.32)	12(±7.03)	11.33(±6.64)	12.21(±6.06)	0.40NS
COD	27.06(±34.00)	35.48(±48.07)	35.13(±57.67)	28.77(±22.73)	37.56(±32.98)	0.35NS

*NS – not significant by F test at 5% significance level.

Results on the microbiological analysis for *E. coli* (MPN 100 mL^{-1}) were 84.27 (supply), 47.27 (D40), 30.16 (D60), 77.14 (D80) and 53.16 (D100). In fact, these results failed to show any significant difference between the water supply and the effluents of different treatments and between the latter. CONAMA Resolution 357 classifies as Class 2 the fresh-water used in aquaculture activity. According to the above standard, thermo-tolerant coliforms should not exceed 1000 MPN by 100 mL[22]. In current research, results for *E. coli* comply with Brazilian legal demands.

Results for pH were 7.71 (supply), 7.59 (D40), 7.59 (D60), 7.52 (D80) and 7.61 (D100). Further, pH failed to show any significant difference when water supply is compared to effluents from different treatments and between themselves.

Kimpara[26] investigated the quality of water supply and that of effluents originated from *M. amazonicum* culture at densities 10/m², 20/m², 40/m² and 80/m². In this particular research, pH of water supply was significantly higher (pH 7.2) than value of effluents from all treatments (pH 6.7), although there was no significant difference among treatments. Since the parameter was determined during the morning, between 6:30 AM and 7:30 AM, the author accounts this fact to the respiration process of the organisms in the pond during the night.

According to Valenti[27], water used in shrimp breeding should be slightly alkaline, with pH between 7.0 and 9.0. Further, pH values between 7.0 and 8.5 are the best for *M. rosenbergii* culture[28]. No information is extant for the most adequate range in the case of *M. amazonicum*. According to CONAMA Resolution 357, pH should range between 6.0 and 9.0. Consequently, the variable complies with recommended values[22].

Concentrations of dissolved oxygen (mg L^{-1}) were 8.60 (supply), 8.27 (D40), 8.43 (D60), 8.43 (D80) and 8.38 (D100). When water supply and treatments were compared and treatments were compared among themselves, the results had no significant difference. Kimpara[26] verified that the water supply's DO was significantly higher than that in the effluents of the ponds throughout the culture. Since collection was undertaken between 6:30 and 7:30 AM, the author accounts the above result to the respiration process at night by organisms in the pond, simillar to the one described for the pH values

Besides respiration, the decomposition of organic matter in the pond bottom consumed oxygen in the water. Consequently, it is highly important to take into account the quantity and quality of feed given to shrimps. Zimmermann[29] recommends dissolved oxygen ranging between 3 and 7 mg L^{-1} for *M. rosenbergii* culture.

CONAMA's Resolution 357 states that in every sample DO should not be less than 5 mg L^{-1}, and thus, results of water supply and from effluents at the final stage of culture comply with current legislation[22].

Mean temperature values (ºC) were 27.13 (supply), 27.63 (D40), 27.41 (D60), 27.47 (D80) and 27.52 (D100). Water temperature did not show any significant difference when water supply and effluents from the different treatments and treatments among themselves were compared. Results for this variable are adequate for freshwater shrimp culture since, according to Food and Agriculture Organization[28], values were between 25 ºC and 32 ºC, or rather, according to Brazilian legislation. According to CONAMA's Resolution 357, effluents may be only released if their temperature is less than 40 ºC[22].

Results of total suspended solids analyses (mg L^{-1}) were 0.06 (supply), 0.07 (D40), 0.06 (D60), 0.06 (D80) and 0.06 (D100). These results showed no significant difference when the water supply was compared to the effluents of the different treatments and the treatments among themselves. Kimpara[26] found that, besides water supply, solids levels in effluents are affected by feeding management, which must be determined to decrease the quantity of particles in the effluent. According to CONAMA's Resolution 357, the highest level for the variable is 500 mg L^{-1} (22).

Increase in nitrogen compounds concentration is related to the addition of feed in the ponds. Ammonia, nitrite and nitrate are formed from the degradation of organic wastes (non-consumed feed, feces and others) in the sediment. Nutrients are an asset to primary production with the grow-out of phytoplankton and the increase of organic compounds in the suspended solids[26].

Mean concentrations for total nitrogen (mg L^{-1}) were 0.44 (supply), 0.45 (D40), 0.48 (D60), 0.48 (D80) and 0.46 (D100). According to the results, there was no significant difference when water supply and effluents from the different treatments and treatments among themselves were compared. There was no rise in the variable's concentration when quantity of feed was increased. This fact is probably due to nitrogen, which was incorporated to the plankton, to the shrimps' biomass and to that still in the sediment.

Since several researchers[29-32] agree that approximately 90% of total nitrogen in water consists of ammonia nitrogen, the concentration of the latter compound was estimated. This variable and total nitrogen did not show any significant difference when water supply and water from effluents of treatments and the treatments among themselves were compared.

Whereas results for nitrite (mg L^{-1}) were 0.019 (supply), 0.01 (D40), 0.01 (D60), 0.01 (D80) and 0.01 (D100), those for nitrate (mg L^{-1}) were 0.92 (supply), 0.74 (D40), 0.46 (D60), 0.59 (D80) and 0.68 (D100). Nitrite and nitrate did not have any significant difference when water supply and the water from effluents of different treatments and treatments among themselves were compared. Similar results were obtained by Kimpara[26], who found that there was no significant difference among the concentrations of total nitrogen, ammonia, nitrite and nitrate among treatments and among treatments themselves and the water supply. However, when Ziemann et al.[33] analyzed the affluent water quality and the water effluent of fresh-water fish and shrimp ponds and that of seawater fish and shrimp, they discovered that effluent had a lower quality than that of the affluent in most parameters analyzed. In fact, concentrations of suspended material and pigments in the effluents of freshwater fish and shrimps were higher. Nitrite and nitrate concentrations were contrastingly lower in effluents than in affluents. According to the authors above, results show that in some cases aquaculture ponds function as nutrient-absorbing treatment systems. Nitrite and nitrate decrease is probably related to an increase in algae, which, in their turn, are associated with an increase in pigment and water turbidity concentrations.

According to CONAMA's Resolution 357, the border concentration of total ammonia nitrogen for Class 2 varies between 0.5 and 3.7 mg L^{-1} and is dependent on pH value. Since pH varied between 7.52 and 7.71, the border concentrations for ammonia nitrogen varied between 1.0 and 2.0 mg L^{-1}, whereas border concentrations nitrite and nitrate were 1.0 mg L^{-1} and 10.0 mg L^{-1}, respectively. Concentrations for nitrogen compounds comply with the Resolution[22].

Mean BOD concentrations (mg L^{-1}) were 8.44 (supply), 11.75 (D40), 12.00 (D60), 11.33 (D80) and 12.21 (D100). Results fail to show any difference when water supply and effluents of different treatments and among treatments themselves were compared. However, the results show that, since there is no significant release of organic matter in the environment, it may be accumulated within the ponds, in the shrimps' biomass and that of other organisms or in the sediment. Kimpara[26] reported similar results for BOD.

CONAMA's Resolution 357 establishes maximum BOD concentrations of up to 5 mg L^{-1} for Class 2 waters. Concentrations could exceed this value in case of the recieving body's self-cleansing capacity shows that the minimum dissolved oxygen predicted concentrations would not comply with CONAMA[22]. However, since results were over 5 mg L^{-1}, conclusion may not be reached since they depended on the characteristics of the receiving body.

COD mean concentrations (mg L^{-1}) were 27.06 (supply), 35.48 (D40), 35.13 (D60), 28.77 (D80) and 35.56 (D100). Results did not show significant difference when water supply and effluents of different treatments and treatments between themselves were compared. Kimpara[26] produced similar results and showed that there was no significant difference between water supply and treatments for this variable.

During harvest, the pond was net-emptied of shrimps and samples of pond effluent were collected at the initial 1/3, middle 1/3 and final 1/3 phases of each pond's harvest. Table 3 shows mean values at harvest and its standard deviation.

Table 3. Mean values ± standard deviation of variables of final grow-out ponds' effluents during harvesting: initial 1/3, middle 1/3 and final 1/3 of harvest in each pond, UNESP Aquaculture Center, (2006)

Analyzed variables	Initial 1/3	Middle 1/3	Final 1/3	F_harvest
E. coli	17.26(±13.56)AB	15.38(±9.5)B	34.10(±20.43)A	3.76**
pH	7.20(±0.56)	7.22(±0.51)	7.22(±0.50)	0.00NS
Dissolved Oxygen	4.81(±0.37)	4.67(±0.39)	4.39(±0.36)	2.97NS
Temperature	20.82(±1.94)B	22.18(±2.31)AB	23.74(±2.64)A	3.57**
Total Solids	0.052(±0.00)A	0.050(±0.00)B	0.047(±0.00)C	15.52**
Nitrate	1.00(±0.74)	0.72(±0.61)	0.33(±0.51)	3.17NS
Nitrite	0.01(±0.01)	0.01(±0.01)	0.01(±0.01)	0.07NS
Total Nitrogen	0.27(±0.04)B	0.30(±0.05)AB	0.39(±0.17)A	4.62**
Chemical Oxygen demand	43.00(±54.63)	53.56(±42.73)	82.67(±90.93)	0.81NS
Biochemical Oxygen Demand	11.89(±4.04)	13.33(±6.58)	16.67(±6.20)	1.65NS

Means with the same letter do not differ significantly between themselves at 5% probability by Tukey's test. ** - significant by F test at 5% level of probability; NS – not significant by F test at 5% level of probability.

Highest concentration of *E. coli* at the harvest phase was significantly higher when compared to the 1/3 middle phase. This was probably due to sediment suspension and to organisms at the pond's bottom.

Further, no significant difference in pH and dissolved oxygen occurred at any time during harvest.

Water temperature significantly increased during harvest. Kimpara[26] verified that surface water temperature was always higher than that at the pond bottom. Consequently, when emptying process started through the drain tubes at the bottom, water was drained from the bottom upwards. Since the bottom water was first released, its temperature was lower than the surface.

Concentration of total solids significantly decreased during harvest. This fact may be due to the initial phase with a greater accumulation of mud near the drain tubes, which release a higher quantity of total solids in the environment when the draining of the pond water starts.

Whereas total nitrogen concentrations increased significantly during harvest, the concentrations of nitrite and nitrate did not have any significant difference at any time.

Mean BOD and COD concentrations during harvest did not have any significant difference.

Variables DO and BOD during harvest produced results that failed to agree with legislation[22]. However, the Resolution allows excess of BOD concentrations of 5 mg L^{-1} when an analysis of self-cleansing of the receiving body shows that minimal concentrations of expected DO would be complied with.

Oxygen concentration varies throughout the day: due to the respiration process by animals during the night; it is lower in the morning but higher in the evening. This is due to the photosynthesis by planktons. Water renewal and harvest, therefore, should be undertaken at the end of the evening. Aerators should be established so that the demands of the Resolution could be fulfilled.

The other variables showed results according to Resolution 357 of CONAMA[22].

CONCLUSION

Since in the final grow-out phase no significant difference occurred between the water supply and effluents, low potential environmental impact in the grow-out phase of *M. amazonicum* is indicated. There was no significant difference between the different stocking densities. Further, intensification of culture did not cause any change in water up to a density of 100 juveniles specimens/m^2. Effluent of the final 1/3 during pond emptying in harvest showed the highest potential impact in the receiving bodies' water quality.

Emphasis should be given to research made during the final grow-out phase with total water recirculating. This fact lessens the environmental impact caused by the water quantity used by kilo of biomass of shrimps produced.

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