Effect of the Amazon River prawn Macrobrachium amazonicicum culture intensification on ponds hydrobiology.

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ABSTRACT: Effect of the Amazon River prawn Macrobrachium amazonicum culture intensification on ponds hydrobiology. Aquaculture intensification has been reported to cause a negative impact on surrounding water bodies. However, it may increase environmental and economical sustainability in farming systems if properly planed. This study was conducted to evaluate the effect of intensification of the Amazon River prawn culture on some water pond characteristics. Twelve 0.01 ha earthen ponds previously limed and fertilized were stocked at 10, 20, 40 and 80 Macrobrachium amazonicum post-larvae/m². A randomized-complete-blocks design with 4 treatments (stocking densities) and 3 replicates was used. Water temperature and dissolved oxygen were measured daily, while pH, conductivity, transparency and water flux were measured weekly. N-Ammonia, N-Nitrite and alkalinity were measured every 15 days. Water samples were taken in the morning (07h30 to 08h30) and in the afternoon (16h30 to 17h00). Rearing cycle was 165-days. In general, water variables presented similar values in all treatments. Dissolved oxygen and pH mean values were significantly higher and mean N-ammonia concentration was significantly lower in the afternoon. Parameter values were within the ones defined as adequate for general aquaculture. Cluster analysis showed no trend to grouping by treatment, which suggests that stocking density was not the major influencing factor on water characteristics. This indicates that the production system used in this experiment presents high capacity to assimilate allochthonous material (such as feed and fertilizers) without deteriorating water quality. Hence, it seems that semi-intensive earthen ponds have internal mechanisms to preserve their stability as occurs in natural aquatic ecosystems. Therefore, M. amazonicicum may be farmed at high densities (up to 80 post-larvae/m²) in a semi-intensive system, with high productivity and no significant changes in the main water variables. In addition, water can also be used more appropriately in high densities, reducing the volume used by unit of produced biomass.

Key-words: Macrobrachium amazonicum, hydrobiology, water quality, aquaculture intensification, stocking density.

RESUMO: Efeito da intensificação do cultivo do camarão-da-amazônia Macrobrachium amazonicum na hibrobiologia dos viveiros. A intensificação da aquicultura pode aumentar o impacto negativo nos corpos de água receptores dos efluentes. No entanto, esta pode aumentar a sustentabilidade ambiental e econômica dos sistemas de produção se for bem planejada. Este trabalho teve como objetivo avaliar o efeito da intensificação do cultivo do camarão-da-amazônia nas características da água de viveiros. Doze viveiros de fundo natural com 0.01 ha, submetidos à calagem e à fertilização orgânica, foram estocados nas densidades de 10, 20, 40 e 80 pós-larvas de Macrobrachium amazonicum/m². O delineamento experimental foi em blocos totalmente casualizados com quatro tratamentos e três repetições. A temperatura e o oxigênio dissolvido foram determinados diariamente. Foram coletadas amostras de água semanais para determinar o pH, a condutividade, a transparência e o fluxo de água e amostras quinzenais para análise de N-Amônia, N-Nitrito e alcalinidade total. O cultivo durou 165 dias. Em geral, as variáveis avaliadas apresentaram valores semelhantes em todos os tratamentos. Valores médios de oxigênio dissolvido e pH foram significativamente superiores à tarde, enquanto a concentração de N-amônia foi significa-
Effect of the Amazon River prawn Macrobrachium...
variables effects on this species is available. According to Boyd & Zimmerman (2000), limnological variables that often cause negative effect on freshwater prawns are: dissolved oxygen, pH, N-ammonia and N-nitrite. On the other hand, intensification may affect alkalinity, conductivity, transparency, and it requires higher volumes of water. Therefore, the objective of this study was to evaluate the hypothesis that intensification of the Amazon River prawn growth-out in a semi-intensive system changes ponds hydrobiology.

Material and methods

This study was conducted at the Crustacean Sector, Aquaculture Center, São Paulo State University - Caunesp, Jaboticabal, São Paulo State, Brazil. The experiment was carried out in twelve 0.01 ha-earthen ponds, approximately 1 m deep, during 165 days. Ponds were drained and air-dried. Afterwards, they were limed (1000 kg hydrated lime/ha), and organically fertilized (3000 kg cattle manure/ha). Then, ponds were filled with water from an upstream dam after passing through a gravel filter.

Ponds were stocked at 10, 20, 40 and 80 M. amazonicum post-larvae (PL)/m² (0.01 g) from Caunesp broodstock. The parental males and females were provenient from Santa Bárbara, Pará State, Brazil (01°13’25”S, 48°17’40”W) in 2001. A randomized-complete-blocks design with 4 treatments (stocking densities) and 3 replicates was used.

Prawns were fed a commercial diet (Supra® - Maringá, Paraná state, Brazil) with 40% crude protein in the first month and a 37% crude protein (Laguna® CMS 37, Socil - Descalvado, São Paulo state, Brazil) diet from the following months. Diet was divided in 2 equal quantities, and was offered to animals at 07h30-08h30 and at 16h00-17h00. Daily feeding rates in each pond was 2.5 g/m² in the first month. From the second month on, daily feeding rates ranged from 10 to 3% of prawn biomass, decreasing as prawns grow. Feeding quantity was reduced by half when dissolved oxygen levels were between 2.5 and 3.5 mg/L in the morning and it was suspended when levels were below 2.5 mg/L. In these cases, a B-500 AQUAHOBBY (Bernauer Aquacultura S.A. - Blumenau, Santa Catarina state, Brazil) emergency aerator was used. Mean daily feed supplied and prawn productions by density were 45, 53, 63 and 102 kg/ha/day and 508, 875, 1,283 and 2,051 kg/ha, respectively for 10, 20, 40 and 80 PL/m². Survival was about 70% in all ponds.

Water temperature and dissolved oxygen were measured daily; pH, conductivity, transparency and water flux were measured weekly, while N-Ammonia, N-Nitrite and alkalinity were measured every 15 days. Water samples were taken in the morning (07h30 to 08h30) and in the afternoon (16h30 to 17h00). Water temperature was measured using index thermometers at surface and bottom of one pond, as no difference was detected among ponds in previous studies. An YSI model 55 oximeter and an YSI model 63 multiparameter sonde (Yellow Spring Instruments, Yellow Springs, OH, USA) were used to determine dissolved oxygen and pH and conductivity levels, respectively. Transparency was measured at 17h00, using a Secchi disk. N-Ammonia concentration was determined according to Solorzano (1969) and N-Nitrite was measured according to the method described by Bendschneider & Robinson (1952); both are colorimetric methods and absorbances were measured using a Hach DR 2000 spectrophotometer (Hach, Loveland, CO, USA). Alkalinity was determined by titration method, according to Boyd (1984).

Treatments were compared in each period of the day separately. All data were subjected to normality and homocedasticity analysis using Shapiro-Wilk and Brown-Forsythe tests (Sokal & Rohlf, 1995), respectively. When these conditions were satisfied, mean values were compared by two-way ANOVA, F-test. On the contrary, the non-parametric Friedman test (Sokal & Rohlf, 1995) was used. When differences were significative, mean values were compared by Fisher-LSD test. To evaluate the difference between morning and afternoon, the t-test was used when data showed normality and homocedasticity. Otherwise, the Mann-Whitney U-test (Sokal & Rohlf, 1995) was used. A cluster analysis (Statistica v.6 - Statsoft, Inc., Tulsa, OK, USA) was used to order stocking densities. It was assumed as a hypothesis that ponds would be
Results

Water temperature was high and ranged from 24 and 32.5°C in the bottom and between 21 and 34°C in the surface. Minimal mean temperature was 28.5 ± 1.9°C in the bottom and 27.7 ± 1.5°C in the surface of ponds. Maximum mean values were 30.9 ± 2.1°C in the bottom and 29.4 ± 1.8°C in the surface. Dissolved oxygen varied from 1.7 (40/m²) to 8.4 mg/L (10/m²) in the morning, and from 3.7 (20 and 80/m²) to 13.8 mg/L (10/m²) in the afternoon. Morning dissolved oxygen values were significantly lower (Tab. I). Treatment 10/m² differed from treatment 20/m² in the afternoon (Tab. I). Mean pH values were significantly lower in the morning, ranging from 7.20 (10 and 20/m²) to 7.31 (40/m²), and from 8.08 (20/m²) to 8.20 (10/m²) in the afternoon (Tab. I). N-ammonia values differed between treatments 10 and 40/m² in the afternoon (Tab. I). N-ammonia varied from zero (all densities) to 335 μg/L (40/m²) in the morning, and zero (all densities) to 357 μg/L (40/m²) in the afternoon. Morning N-ammonia values were significantly higher, except for treatment 80/m² (Tab. I). Nitrite, total alkalinity, conductivity, transparency and water flux presented similar values in all treatments and periods of the day (Tab. I). Cluster analysis did not group ponds according to stocking densities (Fig. 1).

Figure 1: Cluster analysis of limnological variables: the former numbers are referred to pond numbers and the ones inside parenthesis are referred to stocking densities (10, 20, 40 and 80 PL/m²).
Table I: Minimum and maximum values followed by mean ± standard deviation of limnological variables in M. amazonicum rearing ponds at different stocking densities in the morning and afternoon. (PL = post-larvae)

<table>
<thead>
<tr>
<th>Stocking density</th>
<th>Morning</th>
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<th>Afternoon</th>
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<tr>
<td></td>
<td>10 PL/m²</td>
<td>20 PL/m²</td>
<td>40 PL/m²</td>
<td>80 PL/m²</td>
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<td>20 PL/m²</td>
<td>40 PL/m²</td>
<td>80 PL/m²</td>
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<tr>
<td>Dissolved oxygen</td>
<td>2.3 – 8.4</td>
<td>2.6 – 8.2</td>
<td>1.7 – 7.9</td>
<td>1.8 – 8.2</td>
<td>4.6 ± 1.1a</td>
<td>4.5 ± 1.2a</td>
<td>4.3 ± 1.3a</td>
<td>4.4 ± 1.4a</td>
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<td>pH</td>
<td>6.32 – 8.44</td>
<td>6.28 – 8.49</td>
<td>6.21 – 8.68</td>
<td>6.17 – 8.45</td>
<td>7.20 ± 0.48a</td>
<td>7.20 ± 0.45a</td>
<td>7.31 ± 0.54a</td>
<td>7.24 ± 0.51a</td>
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<tr>
<td>N-NH₃ + N-NH₄⁺</td>
<td>0 – 212</td>
<td>0 – 278</td>
<td>0 – 335</td>
<td>0 – 281</td>
<td>78 ± 59a</td>
<td>80 ± 66a</td>
<td>91 ± 83a</td>
<td>75 ± 83a</td>
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<td>N-NO₂</td>
<td>8.6 – 77.8</td>
<td>14.2 – 116.8 &amp; 7.7 – 87.6</td>
<td>10.6 – 132.2</td>
<td>36.9 ± 7.4</td>
<td>36.3 ± 6.3</td>
<td>37.8 ± 10.8</td>
<td>36.7 ± 6.8</td>
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<tr>
<td>Total alkalinity</td>
<td>28.2 – 66.3</td>
<td>27.5 – 55.2 &amp; 26.5 – 87.6</td>
<td>28.2 – 57.6</td>
<td>47.4 – 154.0</td>
<td>54.1 – 149.0</td>
<td>54.2 – 174.7</td>
<td>69.8 – 139.7</td>
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<td>Conductivity</td>
<td>92.5 ± 16.9</td>
<td>92.1 ± 14.3</td>
<td>93.0 ± 19.0</td>
<td>92.7 ± 14.7</td>
<td>92.5 ± 13.8</td>
<td>37.2 – 18.1 &amp; 42.0 ± 23.1</td>
<td>42.8 ± 21.8</td>
<td>43.9 ± 30.0</td>
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<td>Transparency</td>
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<td>4.4 – 13.8</td>
<td>3.7 – 12.1 &amp; 3.8 – 11.6</td>
<td>3.7 – 13.2</td>
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<td>(cm)</td>
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<td>8.3 ± 2.0bA</td>
<td>7.6 ± 1.8bBC &amp; 7.7 ± 2.1bAC</td>
<td>8.2 ± 2.2bAC</td>
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<td>Water flux</td>
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<td>-</td>
<td>6.76 – 9.41</td>
<td>6.79 – 9.22 &amp; 6.83 – 9.26</td>
<td>6.76 – 9.60</td>
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<td>(L.min⁻¹)</td>
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<td>8.20 ± 0.70b</td>
<td>8.08 ± 0.66b &amp; 8.15 ± 0.71b</td>
<td>8.19 ± 0.73b</td>
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<td>N-NH₃ + N-NH₄⁺</td>
<td>0 – 132</td>
<td>0 – 317</td>
<td>0 – 357</td>
<td>0 – 286</td>
<td>18 ± 23bA</td>
<td>33 ± 56bAC &amp; 53 ± 75bBC</td>
<td>45 ± 73aAC</td>
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<td>(µg.L⁻¹)</td>
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<td>N-NO₂</td>
<td>8.8 – 81.0</td>
<td>14.5 – 139.0 &amp; 6.0 – 128.0</td>
<td>13.9 – 121.4</td>
<td>39.4 ± 19.5</td>
<td>44.5 ± 24.7 &amp; 46.3 ± 25.8</td>
<td>43.2 ± 27.2</td>
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<tr>
<td>Total alkalinity</td>
<td>28.2 – 62.0</td>
<td>28.2 – 96.0 &amp; 28.2 – 82.8</td>
<td>26.5 – 57.6</td>
<td>35.8 ± 6.7</td>
<td>36.9 ± 10.8 &amp; 36.6 ± 9.9</td>
<td>35.7 ± 7.0</td>
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<td>Conductivity</td>
<td>74.4 – 161.3</td>
<td>75.6 – 149.8 &amp; 56.0 – 182.2</td>
<td>71.2 – 147.6</td>
<td>95.9 ± 15.6</td>
<td>94.8 ± 13.6 &amp; 95.4 ± 19.7</td>
<td>96.1 ± 14.0</td>
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<td>(µS.cm⁻¹)</td>
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<tr>
<td>Transparency</td>
<td>40.0 – 90.0</td>
<td>40.0 – 75.0 &amp; 35.0 – 85.0</td>
<td>25.0 – 75.0</td>
<td>62.2 ± 10.4</td>
<td>59.3 ± 8.6 &amp; 60.9 ± 11.7</td>
<td>60.1 ± 9.6</td>
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<tr>
<td>Water flux</td>
<td>2.00 – 19.00</td>
<td>1.50 – 16.00 &amp; 1.0 – 45.00</td>
<td>0 – 19.00</td>
<td>6.80 ± 3.54</td>
<td>6.66 ± 3.01 &amp; 5.97 ± 2.66</td>
<td>6.47 ± 3.61</td>
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Lower case letters refer to morning and afternoon comparisons obtained by t-test or Mann Whitney U-test; capital letters refer to comparisons among stocking densities in the same period, obtained by ANOVA or Friedman tests, followed by Fisher-LSD test. Means followed by different letters in the same raw differ significantly (P < 0.05).
Discussion

There is no information about the adequate range of water variables for M. amazonicum culture. Thus, as a reference, values recommended for general aquaculture systems (Timmons et al., 2002) and the ones recommended by some authors for M. rosenbergii farming were adopted (Zimmermann, 1998; Boyd & Zimmerman, 2000; New, 2002). Even so, the high survival rate and productivity obtained in this experiment suggest that water variables were acceptable for M. amazonicum development.

Water temperature was high throughout the rearing cycle. It varied within the adequate range for M. rosenbergii, from 26 to 32°C (Boyd & Zimmerman, 2000). Since M. amazonicum is a tropical species and largely occurs in the Amazon Rivers, it may be assumed that water temperature was adequate for its development.

Dissolved oxygen, pH and N-Ammonia varied throughout the day. These parameters are affected by photosynthesis, decomposition and nitrification processes, which occur all over the day. While decomposition and nitrification occur during the whole day, photosynthesis happens only when there is daylight. Thus, during the night, there will be only decomposition and nitrification and then, oxygen will be consumed while inorganic-nitrogen and CO₂ will be released, decreasing pH. Therefore, observed variations reflect the metabolism of pond communities.

Tolerance levels of dissolved oxygen are similar for farmed warm water fish and crustaceans (Boyd & Tucker, 1998). For a complete development, it is recommended levels above 5 mg/L (Timmons et al., 2002). However, M. rosenbergii tolerates levels of 2 mg/L with no stress, and levels below 1 mg/L start to cause mortality (Zimmerman, 1998; Boyd & Zimmerman, 2000). Survival under low dissolved oxygen levels depends on exposure time, health conditions and size of animals, temperature and other environmental parameters (Boyd & Tucker, 1998). In general, mean oxygen levels varied from 7 to 8 mg/L in all treatments in the present investigation. Studies in temperate regions have obtained dissolved oxygen values of 7.5 mg/L (Tidwell et al., 1998), 7.7 ± 0.1 mg/l (Tidwell et al., 2003) and 8.3 ± 0.2 mg/l (Tidwell et al., 2004) in M. rosenbergii grow-out ponds.

Dissolved oxygen is one of the main factors which limit freshwater prawn culture intensification. Increasing populational densities, feeding rates must be increased. As a consequence, respiration of the main species and microorganisms increases, which reduce dissolved oxygen. However, the management adopted in this experiment was efficient, allowing intensification of M. amazonicum culture up to 80 PL/m² (biomass overcome 2,000 kg/ha) with no changes in dissolved oxygen levels. In spite of the significant difference in this parameter in the afternoon in 10 and 20 PL/m² treatments, there is no evidence that this increase was closely related to intensification, since the value observed in 80/m² was quite similar to those found in treatment 10/m². Moreover, all values were similar in the morning. Therefore, semi-intensive grow-out earthen ponds may present mechanisms to prevent dissolved oxygen depletion when community respiration increases.

The optimum pH range for development and sanity of the majority of freshwater animals is 6.5 to 9.0 (Boyd & Tucker, 1998). Timmons et al. (2002) recommend levels of 6.5 to 8.5 for aquaculture, while Boyd & Zimmerman (2000) recommend levels ranging from 7.0 to 8.5 as ideal for M. rosenbergii production. Nevertheless, according to Sandifer & Smith (1985), this species was cultivated in waters with pH ranging from 6.0 to 10.5 with no apparent adverse effect. In the present study, pH ranged from 6.17 to 9.60, but mean values were between 7.20 and 8.20. Despite of some low or high pH values, it probably did not affect prawns development due to the high obtained survival and productivity.

N-Ammonia is the major nitrogen component excreted by crustaceans and also results from organic matter decomposition by microorganisms (Boyd & Tucker, 1998). For each kg of added feed (20-40% crude protein), approximately 30 g of total ammonia are released in the water (Boyd & Tucker, 1998). Therefore, intensification may increase ammonia concentration to toxic levels. Ammonia toxicity depends on the proportion of ionized and non-ionized forms, which depends on pH. Thus, toxic ammonia effects...
would be more harmful during the afternoon, when pH values are higher. On the contrary, it is partially compensated by ammonia absorption which occurs in the same period by photosynthesizer organisms. Timmons et al. (2002) recommend ammonia concentration below 3,000 µg/L in warm water aquaculture, while New (2000) recommends levels below 500 µg/L in pH 9.5 for M. rosenbergii. Obtained values in this experiment were always lower than those recommended values. Thus, it seems this parameter did not affect M. amazonicum development. As it was previously discussed for oxygen and pH, intensification did not raise ammonia values above toxic levels despite the great increase in excretion and input of organic matter as allochthonous feed. Food supply increased from 45 to 102 kg/ha/day without any change in water nitrogen-ammonia concentration. Hence, ponds present mechanisms to efficiently remove ammonia from water column. It may be via photosynthesis, desamonification or nitrification. Studies should be performed to determine the contribution of each of these processes.

N-Nitrite appears in very low concentrations in aquaculture ponds because it is rapidly converted to nitrate due to nitrification (Boyd & Tucker, 1998). Timmons et al. (2002) recommend maintain nitrite level below 1,000 µg/L for aquaculture systems, while New (2002) affirms that concentrations under 2,000 µg/L are adequate for M. rosenbergii. In this experiment, observed levels were lower than these indicated. Low nitrite concentration indicates that released ammonia is rapidly assimilated by phytoplankton and/or nitrification is adequately occurring. Therefore, results suggest that both nitrification and photosynthesis were not negatively affected by intensification.

Alkalinity has an indirect effect on organisms, making pH stable, increasing water fertility and decreasing toxic potential of metals (Boyd & Tucker, 1998). Thus, waters with very low alkalinity (below 20 mg/L CaCO₃) are not adequate for aquaculture. Timmons et al. (2002) recommend alkalinity ranging from 50 to 300 mg/L CaCO₃. On the other hand, Boyd & Zimmerman (2000) and New (2002) recommend values ranging between 20 and 60 mg/L CaCO₃ for M. rosenbergii culture. In this study, alkalinity was always superior to 20 mg/L CaCO₃. This condition was adequate for the biological processes inside the pond. In addition, alkalinity being similar in all treatments indicates that additional carbon added to ponds as prawn feed was not accumulated in the inorganic carbon compartment.

Intensification dramatically increased M. amazonicum productivity, but it required also more addition of feed. Consequently, much more material was added to ponds. So, this could mean an increase in the quantity of ions in the water column provided by decomposition of faeces, uneaten feed and leaching loss. This did not happen, since mean conductivity values were very similar in all treatments. Ions were probably assimilated by live organisms and/or were deposited on pond bottom. Thus, the used farming system probably has assimilated intensification up to 80 PL/m² in a very efficient way.

Transparency is related to turbidity, or to the quantity of suspended materials. For M. rosenbergii culture, it has been recommended transparency values ranging from 25 and 60 cm (Boyd & Zimmerman, 2000; New, 2002). The obtained mean values in this experiment were close to the superior limit value, which indicates that water was relatively transparent, but it was adequate for prawn culture. The higher feed input in culture systems with higher stocking densities did not increase the quantity of suspended materials, nor induced higher primary production. This material was probably incorporated in detritivorous organism and prawn biomass, or it was deposited on the bottom of ponds or converted into gas (as NH₃, CO₂ and N₂) and then exported to the atmosphere.

Mean exchange rate was similar in all treatments, indicating an adequate hydric management. The mean value was 6.5 L/min, which corresponds to a daily exchange rate of 9% considering inflow water as a reference. This value is within exchange rate recommended for freshwater prawn culture that is 3 to 35%/day, according to Zimmerman (1998). However, during the past years, economy in water consumption has been an important concern in aquaculture, and exchange rates should be reduced to minimum values, necessary only to maintain the ponds level by compensating losses from evaporation and seepage (Wickins & Lee, 2002). In this context, the volume of water used per unit of produced
seafood may be an interesting parameter to compare species and production systems (Hargreaves et al., 2002). In this study, approximately 310, 180, 125 and 80 m³ water/kg prawn were used in the stocking densities 10, 20, 40 and 80 PL/m², respectively. It may be concluded that intensification may lead to a more efficient use of water.

Cluster analysis did not group ponds of the same treatments, which may indicate that populational density was not the major influencing factor on group formation. Other factors, such as pond position (wind and sunlight incidence angles), soil characteristics, etc seem to have more influence on water variables than the changes in the management and the increasing in the allochtonous material input due to intensification. This indicates that the production system used in this experiment presents high capacity to assimilate allochtonous material (such as feed and fertilizers) without deteriorating water quality. Robinson et al. (2004) observed no effect of feeding rate on nitrogen waste in freshwater fish ponds, while Southworth et al. (2006) attributed the low levels of total ammonia in intensively fed channel catfish ponds to phytoplankton assimilation. Similar results were obtained by Trott & Alongi (2000) in a tropical mangrove estuary. Hence, it seems that semi-intensive earthen ponds have internal mechanisms to preserve their stability as occurs in natural aquatic ecosystems. Probably, mechanisms of feedback and alternative circuits of recycling nutrients take part in this regulation. Results obtained in this study indicate that M. amazonicum semi-intensive grow-out systems presents great capacity to receive increasing amounts of allochtonous organic matter, which are necessary for intensification. This species may be farmed at high densities (up to 80 PL/m²) and present high productivity (up to 2,000 kg/ha) with no significative change on dissolved oxygen, pH, ammonia and nitrite concentrations, which are some of the most important variables for freshwater prawn culture. Moreover, intensification allows more adequate water use, reducing volume used per produced biomass and thus fitting sustainability.

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