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UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CENTRO DE AQUICULTURA DA UNESP

Balanço de nitrogênio, fósforo e carbono em sistema multiespacial e multitrófico de cultivo de tilápia-do-nilo e camarão-da-amazônia

Fernanda Seles David

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TÍTULO: Balanço de nitrogênio, fósforo e carbono em sistema multiespacial e multitrófico de cultivo de tilápia-do-Nilo e camarão-da-amazônia

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"Queira! Basta ser sincero e desejar profundo. Você será capaz de sacudir o mundo, vai! "

Tente outra vez - Raul Seixas/ Paulo Coelho/ Marcelo Motta

SUMÁRIO

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RESUMO

O presente trabalho visou caracterizar os balanços de nitrogênio (N), fósforo (P) e carbono (C) em sistemas multitróficos e multiespaciais de tilápia-do-nilo e camarão-da-amazônia. Além disso, traz informações sobre o efeito da adição de diferentes substratos nestes sistemas. O delineamento experimental utilizado foi inteiramente casualizado, com três tratamentos e quatro repetições: sem substrato; substrato de manta geotêxtil; substrato de bambu. Os resultados mostram que a dieta é a principal entrada de nutrientes nos balanços (~66% N, ~56% P e ~59% C orgânico), seguida pela água de entrada (~31% N, ~24% P e ~34% C total). A porção de C recuperada nas tilápias foi mais eficaz no tratamento de manta geotêxtil do que no tratamento de bambu. As porções de N e P recuperadas pelos animais não diferiu entre os tratamentos. O sedimento foi o compartimento que mais acumulou nutrientes (~31% N, ~64% P e ~54% CO). A água de saída foi menos impactante com relação ao P nos tratamentos com adição de substratos. A troca de gases entre os viveiros e a atmosfera mostrou o importante papel da desnitrificação ao liberar quantidades representativas de N_2 (~32% N) e também o potencial desse sistema para a absorção de gases do efeito estufa (~6,2% C). O mapeamento de N, P e C em cada compartimento do sistema revelou o destino dos recursos investidos e indicou que o desenvolvimento do perifiton deve ser melhor investigado para melhorar a eficiência do sistema.

PALAVRAS-CHAVE: Balanço de massa, nitrogênio, fósforo, carbono, substrato, IMTA, perifíton.

ABSTRACT

The present study investigated the use of nitrogen (N), phosphorus (P) and carbon (C) in Integrated Multi-Trophic Aquaculture (IMTA) systems with Amazon River prawn and Nile tilapia. In addition, it provides information about the effect of the addition of different substrates in these systems. The experimental design was completely randomized, with three treatments and four replications: control, without substrate (WS); substrate made of geotextile blanket (GS); and substrate made of bamboo (BS). The results showed that diet was the main input of nutrients in the budgets (~ 66% N, ~ 56% P and 59% Organic C), followed by the inlet water (~ 31% N, ~ 24% P and 34 % Total C). The recovered portion of C in tilapias was more effective in GS treatment than in BS treatment. Recovered portions of N and P by reared animals did not differ among treatments. Sediment was the compartment that accumulated more nutrients (~ 31% N, ~ 64% P and ~ 54% OC). The outlet water was less harmful, with regarding to P, in treatments with addition of substrates. Gas exchange between ponds and atmosphere showed the important role of denitrification by releasing representative quantities of N_2 (~ 32% N) and the potential of this system for the absorption of greenhouse gases (~ 6.2% C). The mapping of N, P and C in each compartment of the system allowed the understanding of invested resources destination and pointed out that periphyton development needs further attention to increase system’s efficiency.

KEY WORDS: Mass balance, nitrogen, phosphorus, carbon, substrate, IMTA, periphyton.

CAPÍTULO 1

INTRODUÇÃO GERAL

A aquicultura tem sido o setor de produção alimentícia que mais cresce no mundo, provendo quase 50% do total de pescado que é destinado ao abastecimento global e com estimativa de crescimento de mais 12% até 2030 (FAO, 2014). A expansão da aquicultura é uma necessidade, porém esse intenso desenvolvimento tem sido acompanhado de vários impactos ambientais (Bayle-Sempere et al., 2013). A poluição de corpos d'água pelos efluentes produzidos e o uso ineficiente de recursos naturais são os principais exemplos (Boyd, 2003). Para que estes impactos sejam minimizados, o desenvolvimento do setor deve ser promovido e gerido de forma responsável (Bayle-Sempere et al., 2013).

Ao utilizar de forma racional os recursos naturais, manufaturados e humanos, a atividade torna-se perene e lucrativa (Valenti, 2002) e, se praticada com base científica, pode aumentar o aproveitamento destes recursos. Na tentativa de direcionar a aquicultura para o crescimento sustentável, têm-se buscado alternativas aos modelos tradicionais de cultivo (monocultivos) e a estratégia denominada cultivo multiespacial e multitrófico vem se destacando. Este é um modelo de produção de organismos aquáticos com integração de espécies (Chopin et al., 2001; Neori et al., 2007) de diferentes nichos tróficos (Neori et al., 2004). Assim, os resíduos alimentares de espécies alvo são reaproveitados por espécies co-cultivadas de valor comercial (Reid et al., 2013). A tilápia-do-nilo (*Oreochromis niloticus*) e o camarão-da-amazônia (*Macrobrachium amazonicum*) apresentam características que permitem a exploração de diferentes nichos em viveiros. A primeira, geralmente considerada como espécie alvo em cultivos multitróficos e multiespaciais, nada ativamente na coluna d'água e pode filtrar o plâncton. Em contrapartida, o camarão-da-amazônia tem hábito bentônico e se alimenta principalmente de detritos, sendo considerado geralmente como espécie co-cultivada. A integração dessas duas espécies, portanto, gera produtos diversificados para o mercado aquícola (Ridler et al., 2007) e pode aumentar a eficiência dos sistemas através do reaproveitamento de nutrientes (Castellani & Abimorad, 2012), gerando efluentes menos impactantes e evitando a eutrofização e sedimentação em águas receptoras (Henry-Silva & Camargo, 2006; Kubitza, 2003).

Cultivos multitróficos e multiespaciais podem ser ainda mais eficazes com a introdução de camadas verticais no interior dos viveiros. Estas camadas, conhecidas como substratos, aumentam a área útil dos viveiros e minimizam as interações sociais intraespecíficas das espécies bentônicas (Tidwell & Bratvold, 2005). Isto ocorre pela ocupação da dimensão vertical dos viveiros pelos camarões, melhorando o bem-estar dos animais e possibilitando maior intensificação do cultivo (Tidwell et al., 1999; 2000). Além disso, o uso de substratos possibilita a colonização de perifíton em sua superfície (Ranjeet & Hameed, 2015), criando um novo compartimento no sistema que acumula nutrientes-chave como o nitrogênio, o fósforo e o carbono. Esse novo compartimento acumula energia e nutrientes devido ao processo de fotossíntese e à retenção de partículas suspensas na água. Desta forma, torna esses nutrientes, que estavam em compartimentos não disponíveis, acessíveis para as espécies de cultivo.

Tem sido observado que os substratos melhoram a qualidade da água e reduzem a taxa de conversão alimentar em viveiros de monocultivo de camarões e tilápias (Tidwell et al., 1999; 2000; Azim et al., 2004; Milstein et al., 2008; Ranjeet & Hameed, 2015). Resultados semelhantes também foram observados em sistemas multiespaciais e multitróficos de tilápias e camarões (Uddin et al., 2006; 2007a; 2007b; 2008; Milstein et al., 2008). No entanto, trabalhos na literatura que abordem o balanço de nutrientes-chave em sistemas multiespaciais e multitróficos são escassos.

Trabalhos sobre balanço de nutrientes têm sido pouco explorados pela comunidade científica devido às dificuldades metodológicas, mas têm sido apontados como o principal caminho para a aquicultura atingir a sustentabilidade. A maioria dos trabalhos disponíveis na literatura sobre balanços de nutrientes abordam sistemas de monocultivo de camarões (Thakur & Lin, 2003; Casillas-Hernandez et al., 2006; Van Khoi & Fotedar, 2010; Sahu et al., 2013a; 2013b; Saraswathy et al., 2013; Adhikari et al., 2014) e são considerados incompletos, pois não contabilizam a entrada de nutrientes pela água da chuva e também as trocas gasosas dos viveiros com a atmosfera.

Deste modo, o presente trabalho visou caracterizar os balanços de nitrogênio, fósforo e carbono em sistemas multitróficos e multiespaciais de tilápia-do-nilo e camarão-da-amazônia. Além disso, traz informações sobre o efeito da adição de diferentes substratos nestes balanços, testando a hipótese de que a adição de substratos aumenta a reciclagem de nutrientes em sistemas multitróficos e multiespaciais de tilápia-do-nilo e camarão-da-amazônia e que o tipo de substrato usado afeta este processo.

APRESENTAÇÃO DO TRABALHO

A presente Tese foi dividida em três capítulos redigidos em inglês sob a forma de artigos científicos. Com o intuito de caracterizar os balanços de nitrogênio, fósforo e carbono em detalhes, cada elemento foi analisado de forma independente. No entanto, como os resultados obtidos nos balanços são provenientes de um mesmo cultivo, as seguintes informações disponíveis na metodologia não diferem entre os artigos: delineamento experimental, manejo, qualidade da água de cultivo e despesca. Basicamente, a diferença metodológica entre os balanços consiste nas análises realizadas para quantificar os elementos em cada compartimento do sistema.

Os artigos de nitrogênio e fósforo foram submetidos para as revistas Aquaculture e Journal of the World Aquaculture Society, respectivamente. Portanto, ambos estão apresentados na Tese de acordo com as normas de cada revista. O artigo de carbono será submetido em breve para publicação.

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CAPÍTULO 2

NITROGEN BUDGET IN INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEMS WITH NILE TILAPIA AND AMAZON RIVER PRAWN

Nitrogen budget in integrated multi-trophic aquaculture systems with Nile Tilapia and Amazon river prawn

Abstract

The present work aims to investigate the use of nitrogen (N) in Integrated Multi-Trophic Aquaculture (IMTA) systems with Nile tilapia (*Oreochromis niloticus*) and Amazon River prawn (*Macrobrachium amazonicum*), with and without the addition of different substrates. The experimental design was completely randomized, with three treatments and four replications: control, without substrate (WS); substrate made of geotextile blanket (GS); and substrate made of bamboo (BS). Diet was the major input of N in the system, ranging from (~61 to 71%) followed by inlet water (~26 to 36%). The portion retained in reared animals and periphyton ranged from ~21 to 24% (being ~21 to 22% in fish and prawns). The output compartments that contributed most to the accumulation and release of N_2 were, respectively, sediment (~23 to 38%) and N_2 emitted to atmosphere (~28 to 36%). The mapping of nitrogen in each compartment of the system allowed the understanding of invested resources destination in this prawn-tilapia IMTA system and pointed out that periphyton development needs further attention. The addition of different types of substrates in the system did not improve the recycling of nitrogen and this lack of treatment effects seems to be related with the low periphyton biomass and turnover rate. Additional analyzes to better understand the periphyton development should be performed in further studies. Moreover, some alternatives could be tested to investigate the improvement of nitrogen recycling in this prawn-tilapia IMTA, such as the increase in prawns' stocking density, the addition of other detritivores-iliophagus (mud eating) species, the increase of substrates surface area and/or the reduction of the feed rate in order to force the animals to eat periphyton and wastes.

Key words: Mass balance, nitrogen budget, substrate, IMTA system, periphyton.

Highlights

- This study is the first nitrogen budget in IMTA system with and without substrates.
- The use of periphyton substrates has potential to regenerate lost nutrients in animal biomass, but the addition of substrates (50% of the water surface area) in the IMTA system did not affect nitrogen retention by fish and prawns.
- Substrate-based ponds accumulate sediment with lower organic matter than ponds without substrates, promoting aerobic metabolism pathways.
- The emission of N_2 through bubbling rate removed ~32% of non-recovered nitrogen by reared animals and periphyton.

1. Introduction

Aquaculture is still the fastest growing food-producing sector in the world, providing almost half of all fish for human food (FAO, 2014). The sector will continue expand worldwide to supply the increasing demand for high quality proteins. Nevertheless, this development needs to be promoted and managed in a responsible manner that minimizes negative environmental impacts (Bayle-Sempere et al., 2013). The culture of different species sharing the same pond may optimize the use of space, water and other natural resources. Thus, integrated multi-trophic aquaculture (IMTA) systems represent a new strategy to improve environmental sustainability. This strategy allows a more efficient use of nutrients and produces less waste (Diana et al., 2013). Nevertheless, it is essential to know how nutrients are distributed in the several compartments of aquaculture ponds in order to manage the system and obtain the maximum nutrients concentration in the biomass of target animals.

The IMTA systems explore the synergistic interactions of species with complementary ecosystem functions (Chopin, 2006; 2013). The Nile tilapia (*Oreochromis niloticus*) and the Amazon river prawn (*Macrobrachium amazonicum*) have characteristics that allow such exploitation because of the occupation of different niches in ponds. Tilapia swims actively in the water column and filters plankton, while prawns have benthonic habit and feed mainly on detritus and benthic organisms. In IMTA systems, tilapia may be fed using commercial floating diet, whereas prawns may eat tilapia feces and leftover diet. Such combination represents the farming of a fed species (Nile tilapia) with an extractive species (Amazon river prawn) and takes advantage of the synergistic interactions between them.

The addition of substrates in tilapia-prawn culture ponds may increase the efficiency of the system. Substrates allow prawns to explore vertical dimensions in ponds, increasing useful area for benthic species and reducing agonistic encounters and social interactions (Tidwell et al., 1999; 2000). The substrates also provides space for periphyton settlement, which can absorb nutrients from the system, making them available for the reared species. Some studies have documented the advantages of adding artificial substrates to aquaculture systems (Asaduzzaman et al., 2009; Milstein et al., 2008; Uddin et al., 2008). Nonetheless, these studies do not include nutrient budgets, which are important to understand the efficiency of aquaculture practices.

The improvement of aquaculture efficiency requires detailed knowledge of nutrient cycling in different production systems. The first step to understand this process in ponds is to know the nutrient budgets, which quantifies the key elements in each compartment. This can help to identify the destination of supplied resources and, thus, change practices to enhance the system efficiency. Nitrogen is a key element because it is essential for animal nutrition and for the control of environment pollution (Jimenez-Montealegre et al., 2002). A quantitative understanding of nitrogen budget is a prerequisite to achieve waste reduction (Mariscal-Lagarda and Paez-Osuna, 2014) and decrease the chemical fertilizers dependency (Fernando and Halwart, 2000). Thus, the objective of this work is to describe the nitrogen budget, by characterizing the inputs and outputs and quantifying the nitrogen content in all compartments of prawn-tilapia IMTA systems. In addition, we tested the hypothesis that the addition of substrates increases the retention of nitrogen according to the type of substrate.

2. Materials and Methods

2.1 Experimental design

The experiment was conducted at the Crustacean Sector of the Aquaculture Center, São Paulo State University, Brazil (21°15’22’’S, 48°18’48’’W). Juveniles of *M. amazonicum* (0.03 ± 0.01 g) were stocked in twelve earthen ponds (oxisols, ~0.01 ha and 1 m of water depth) at a density of 21.5 individuals/m^2. After five weeks, juveniles of *O. niloticus* (29.0 ± 1.1 g) were stocked in the same ponds at a density of 1.16 individuals/m^2 beginning the IMTA system. Three treatments were tested on this prawn-tilapia IMTA system to evaluate the use of nitrogen: (1) control, without substrate (WS); (2) substrate made of geotextile blanket (GS); and (3) substrate made of bamboo (BS). Four replicates of each treatment were assigned randomly to the ponds.

2.2 Pond management

We used a system with no water exchange, adding inlet water only to compensate water lost by seepage and evaporation. This water receives high loads of nutrients from local fish farming and, thus, it is nutrient-rich. Except for the control group, each pond received substrates equivalent to 50% of its water surface area (Tidwell et al., 2004). The substrates were arranged vertically inside the ponds with the aid of floating plastic bottles. Additional substrates were installed inside net fences to avoid predation and were used for periphyton analysis. All ponds were fertilized with urea and simple superphosphate at the rate of 2 kg N/ha and 8 kg P_2O_5/ha. After 10 days of pond fertilization, when a dense phytoplankton and periphyton population had developed in the systems, prawns and tilapias were stocked according to the experimental design.

The same feed regime was used in all ponds. Prawns were fed with pelleted diet (35% crude protein) at a rate of 10% of body weight in two feeding times per day only until stocking tilapia. After that, prawns were not fed. Tilapias were fed daily with a pelletized diet (40% crude protein in the first month and 28% for the rest of the culture period) at a rate of 4-2% of tilapia biomass that was adjusted monthly. Daily feed was divided in two equal portions, at 12:00 and 16:00 hours. The amount of feed not consumed by tilapias 15 minutes after each feeding time (leftover) was removed from the ponds and discounted from values of supplied diet. Every month, 30 tilapias and 50 prawns were randomly sampled to take biometric measurements and to recalculate feed quantity. After the biometric analysis, the animals returned to the ponds.

2.3 Pond water quality

To characterize the culture, the following variables of water quality were measured biweekly: total ammonia nitrogen (APHA, 2005; 4500-NH_3 F. Phenate method), nitrite nitrogen colorimetric (APHA, 2005; 4500-NO_2^- B. Colorimetric method), nitrate nitrogen (APHA, 2005; 4500-NO_3^- E. Cadmium reduction method), transparency (Boyd, 1979; Secchi disc) and total suspended solids (APHA, 2005; TSS dried at 103-105ºC). Temperature and dissolved oxygen (DO) were monitored daily and pH was measured weekly. These parameters were determined in situ (at 20-30 cm below the water surface) at 08:00 hours using a digital YSI professional plus (Yellow Springs Instruments, Yellow Springs, OH, USA). Water quality parameters are given in Table 1. Emergency aerators were used when DO declined bellow 1.5 mg/L.

Table 1. Means (±SD) of water quality parameters in IMTA* system with *O. niloticus* and *M. amazonicum* obtained from the following treatments: without substrate (WS), geotextile substrate (GS) and bamboo substrate (BS). Minimum and maximum values reached are shown inside parentheses.

Water quality parameters	Treatments		
	WS	GS	BS
T (°C)	27.1 ± 0.9 (20.5 – 29.4)	27.1 ± 0.9 (20.5 – 29.3)	27.1 ± 0.9 (22.7 – 29.5)
DO (mg/L)	4.5 ± 1.3 (0.8 – 9.4)	4.0 ± 1.5 (0.8 – 9.2)	4.1 ± 1.2 (0.8 – 9.0)
pH	7.87 ± 0.45 (7.18 – 9.13)	7.88 ± 0.15 (7.21 – 8.79)	7.71 ± 0.20 (7.14 – 8.25)
$N\text{-}NH_3$ (µg/L)	138.4 ± 35.2 (16.6 – 561.1)	143.5 ± 30.5 (25.5 – 465.2)	109.3 ± 23.6 (6.8 – 303.7)
$N\text{-}NO_2^-$ (µg/L)	8.1 ± 3.6 (0.2 – 69.4)	11.2 ± 3.2 (0.6 – 70.7)	5.8 ± 1.6 (0.4 – 21.1)
$N\text{-}NO_3^-$ (µg/L)	53.0 ± 27.0 (1.8 – 241.8)	85.6 ± 25.3 (1.5 – 270.3)	43.2 ± 24.5 (1.4 – 168.9)
Transparency (cm)	34.6 ± 2.1 (8 - 74)	39.5 ± 4.7 (13 - 82)	35.0 ± 4.7 (13 - 74)
TSS (mg/L)	32.0 ± 23.1 (8.1 – 85.1)	32.2 ± 24.6 (9.3 – 76.9)	29.7 ± 19.1 (9.7 – 57.4)

*IMTA: Integrated Multi-trophic Aquaculture.

2.4 Harvest

The experiment ended on the 140th day because of the cold weather (mid-autumn). All ponds were dried, totally harvested and all animals were counted. To determine individual weight, we analyzed all fish and a random sample of 10% of all prawns from each pond. The animals were weighted (Precision Balance Mate-AS2000C; 0.1 g precision) and measured (wood caliper; 1 mm precision). Survival, mean weight and productivity were determined. These production variables are given in Table 2.

Table 2. Means (±SD) of production variables in IMTA* system with *O. niloticus* and *M. amazonicum* obtained from the following treatments: without substrate (WS), geotextile substrate (GS) and bamboo substrate (BS). Different letters in the same line indicate significant differences between treatments ($P<0.05$).

Production variables	Treatments		
	WS	GS	BS
O. niloticus			
Survival (%)	79.3 ± 7.4	86.7 ± 1.2	88.0 ± 3.2
Mean wet weight (g)	521.7 ± 42.8	493.2 ± 37.8	474.7 ± 58.5
Productivity (kg/ha)	4,794.4 ± 195.6	4,988.3 ± 404.3	4,852.6 ± 460.7
M. amazonicum			
Survival (%)	76.4 ± 4.4	64.5 ± 17.0	74.0 ± 9.3
Mean wet weight (g)	2.7 ± 0.2^{b}	3.5 ± 0.4^{a}	3.1 ± 0.5^{ab}
Productivity (kg/ha)	435.8 ± 15.4	483.1 ± 115.3	480.8 ± 29.1

*IMTA: Integrated Multi-trophic Aquaculture.

2.5 Nitrogen budget

To calculate nitrogen (N) budget we divided the system in compartments of input (diet, inlet water, rainwater, fertilizer, N_2 absorbed, stocked fish, and stocked prawns) and output (outlet water, N_2 emitted, periphyton, harvested fish, harvested prawns, dead fish, and sediment). Subtracting the total input loads from the total output loads, we determined the unaccounted portion of N.

Nutrient input and output in water was calculated by multiplying total N concentration by total water volume. Total N concentration in the inlet water is related to the day of fish stocking (beginning of IMTA system) and in the outlet water to the day before harvest. The total N content in water was determined according to persulfate method (APHA, 2005; 4500-N C.). The inlet water volume is the total amount of water used to fill the ponds plus the volume lost by evaporation and seepage. Rainwater samples were collected five times during the experiment. We analyzed all samples and used a mean value of total N concentration. Rainwater volume was calculated using rainfall data from the Agrometeorological Station of UNESP Jaboticabal, Brazil. Total precipitation volume in the culture period (measured in L/m^2) was adjusted for each pond area, and then, multiplied by the mean total N concentration in rainwater. For chemical fertilization, we used the N concentration provided by the manufacturer.

Samples of stocked and harvested animals were analyzed in triplicate (APHA, 2005; 4500- Norg) and the mean of total N concentration was multiplied by the entire biomass of animals. All dead fish were removed from ponds throughout the experiment. Therefore, it was regarded as an outlet compartment. The entire mass of dead fish in each pond was multiplied by the total N content retained in fish. Nutrient input through feed was calculated by multiplying the total N concentration in diet (APHA, 2005; 4500- Norg) by the entire amount of diet supplied.

Gaseous nitrogen (N_2) was estimated by two analyses: diffusive and bubbling (Matvienko, et al., 2001). For the first, we evaluated the diffusion at the air-water interface by the balance method with the aid of a diffusion chamber. This methodology allows a partial equilibrium between the gas dissolved in the water and the gas inside the chamber through the diffusion of gas to the water (absorption) or from the water (emission). Thus, a diffusion chamber was placed in contact with the surface of the water and one liter of air, collected as close as possible to the air-water interface, was placed inside the chamber. Samples of air inside the chamber were collected monthly in periods of 0, 1, 2 and 4 minutes to determine the gas flow, during the day (between 10:00 – 12:00 hours) and at night (between 22:00 – 24:00 hours). To capture the bubbles (emission), glass fiber funnels suspended by floats were installed on the surface of ponds. We connected a graduated bottle at the extremity of the funnels to trap the bubbles released within 24 hours. The air samples from both methods were placed in transfer tubes for analysis by gas chromatography with TCD detector (Thermal Conductivity Detector from Construmaq, São Carlos, Brazil). The final value corresponds to the sum of absorption (input) or emission (output) daytime and nighttime throughout the experiment.

Nitrogen retained in the periphyton was analyzed by samples of 10 cm width by 20 cm height collected from the additional substrates inside the net fences (between 20 and 40 cm from the water surface). We extracted the periphyton from the substrates using an ultrasonic homogenizer (USC – 750, Unique) according to Thompson et al. (2002). The samples were dried (AOAC, 1995 - 934.01) and the total N content was analyzed by combustion in high temperature and conversion of samples into gases (CHNS Elementar - Vario Macro Cube with Thermal Conductivity Detector sensor). From the dry mass sample, we estimated the entire mass adhered on the substrates. Then, to calculate the retained N in the substrates, we multiplied the entire periphyton mass by total N content.

Sediment samples were collected by tripton samplers (six 1.876 L PVC tubes, with 9.7 cm diameter and 25.4 cm long to 15 cm depth). One tripton sampler was placed on the bottom of each pond for 48 hours biweekly. Samples were dried (AOAC, 1995- 934.01) and analyzed to determine the total N content (APHA, 2005; 4500- Norg). From the total amount of sediment accumulated in 48 hours in the area of the tripton's sampler (~0.045 m²), we estimated the total amount of sediment accumulated in the pond. Then, to calculate the total N in sediment, we multiplied the accumulated mass by N content in the sample. Organic matter was determined by incinerating samples in a muffle furnace at 550º C (AOAC, 1995 - 920.153).

2.5 Data analyses

Survival data was square root arcsine transformed prior to analysis. All data were tested for normality (Shapiro–Wilk) and homoscedasticity (Levene). As both conditions were satisfied, analysis of variance (One-Way ANOVA) was applied to verify the differences in variables of water quality, productions, and compartments of nitrogen budget. Productivity data of prawns were not normal and, therefore, were analyzed with Kruskal-Wallis test. Statistical analyses were carried out in R software (version 0.98.945) and the level of significance considered was $\alpha = 0.05$. When significant differences were detected among treatments, means were compared by Tukey's test.

3. Results

Total nitrogen loads and the comparisons among treatments are shown in Table 3. Total nitrogen percentages are represented as flow diagrams in Figures 1, 2, and 3. Total input values were higher than the output in treatments without substrate (WS) and substrate made of bamboo (BS). In case of substrate made of geotextile blanket (GS), the unaccounted nitrogen was identified in the input. Diet was the major input of nitrogen in all treatments, ranging from ~61 to 71%, followed by inlet water, ranging from ~26 to 36%. The other input compartments together ranged from 1.5 to 3.6%. No significant differences were found in the input compartments.

Nitrogen budget revealed that WS had the major portion of nitrogen inputs deposited in the sediment (~38%), whereas GS and BS had gas emission, ~36% and ~28% respectively. The organic matter (OM) accumulated in the sediment accounted for ~34 to 43% of the dry matter and was significantly lower in periphyton-based treatments. Nitrogen retained (recovered) in reared animals and periphyton ranged from ~21 to 24% (being 19 to 19.7% for fish; 2.2 to 2.5% for prawns; 0.7 and 2.1% for periphyton). Considering the diet as the only input source, the portion of nitrogen recovered by fish was ~25 to 31%. Outputs in the outlet water during harvest ranged from ~5 to 6%. Nitrogen in dead fish ranged from ~2 to 3%. The output compartments showed statistical difference only in periphyton; the GS retains more nitrogen than BS. The final budget showed that the unaccounted nitrogen ranged from 0.4 to 19.8% among treatments; it was significantly higher in BS and lower in GS; both treatments did not differ from control group.

Table 3. Means (±SD) of nitrogen budget in IMTA* system with *O. niloticus* and *M. amazonicum* obtained from the following treatments: without substrate (WS), geotextile substrate (GS) and bamboo substrate (BS). Different letters in the same line indicate significant differences between treatments (*P*<0.05).

Compartments (kg/ha)	**Treatments**		
	WS	**GS**	**BS**
Input			
Diet	368.0 ± 36.8	355.8 ± 20.8	345.5 ± 31.2
Inlet water	135.2 ± 20.2	160.7 ± 52.1	203.2 ± 70.4
Rainwater	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Fertilizer	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
N_2 absorbed	6.1 ± 6.9	0.3 ± 0.6	12.5 ± 8.0
Stocked fish	5.4 ± 0.4	5.4 ± 0.9	5.3 ± 0.3
Stocked prawns	0.2 ± 0.01	0.1 ± 0.02	0.1 ± 0.01
Output			
Outlet water	29.1 ± 7.7	30.8 ± 6.5	26.6 ± 4.7
N_2 emitted	162.5 ± 70.7	191.5 ± 29.5	160.0 ± 82.9
Periphyton	-	11.4 ± 2.0^{a}	3.9 ± 1.3^{b}
Harvested fish	98.4 ± 11.0	105.8 ± 16.7	112.3 ± 11.9
Harvested prawns	11.6 ± 2.7	12.2 ± 4.0	14.5 ± 0.7
Dead fish	16.5 ± 5.5	10.9 ± 1.4	10.1 ± 2.6
Sediment	197.1 ± 62.9	176.4 ± 71.0	128.9 ± 36.9
Unaccounted			
Input - Output	1.89 ± 81.8^{ab}	-14.2 ± 36.6^{b}	112.6 ± 59.3^{a}

*IMTA: Integrated Multi-trophic Aquaculture.

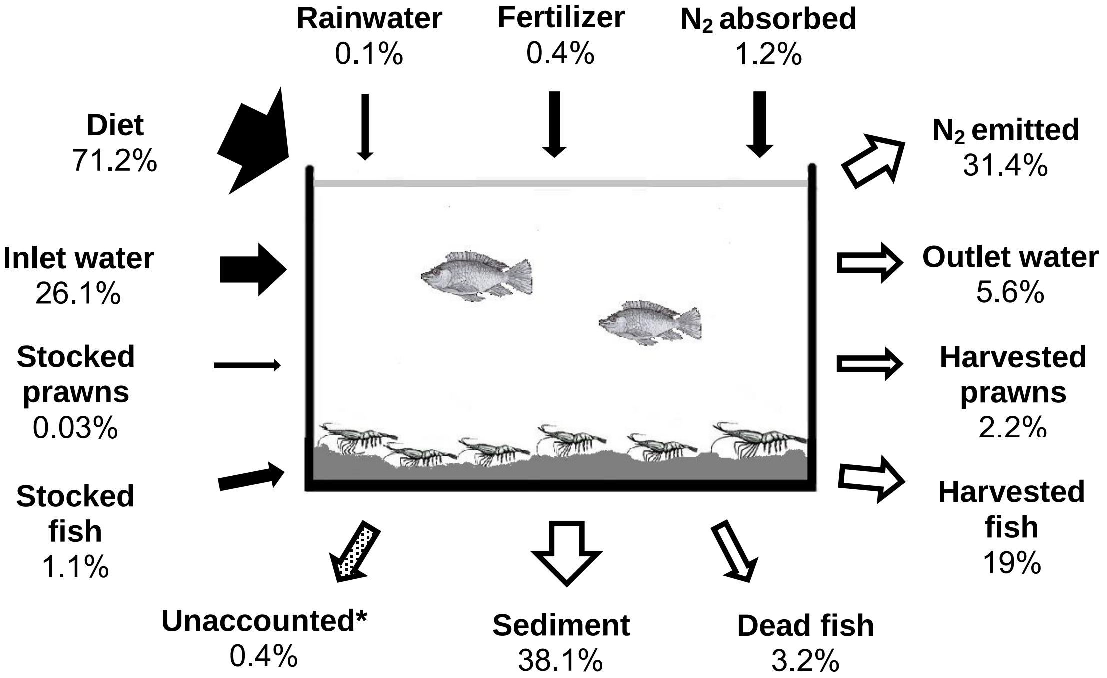

Figure 1. Nitrogen budget in Integrated Multi-Trophic Aquaculture system with *O. niloticus* and *M. amazonicum* in treatment without substrate (WS). Values are shown in percentages based on the total input to pond. *Indicate significant differences ($P<0.05$) between treatments.

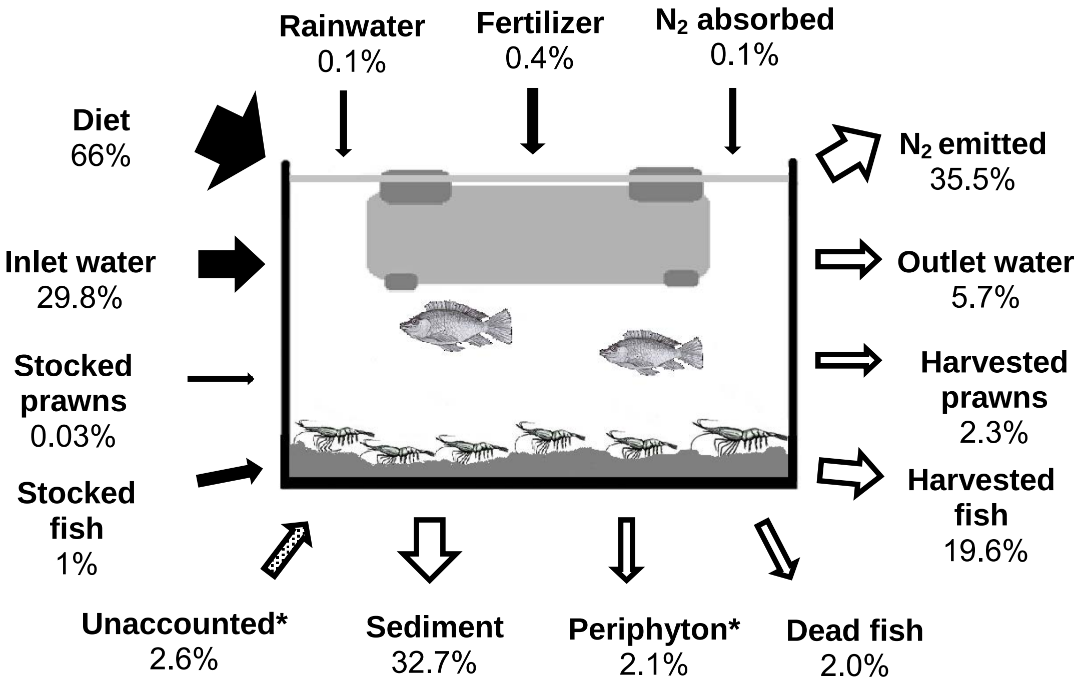

Figure 2. Nitrogen budget in Integrated Multi-Trophic Aquaculture system with *O. niloticus* and *M. amazonicum* in treatment with substrate made of geotextile blanket (GS). Values are shown in percentages based on the total output to pond. *Indicate significant differences (P<0.05) between treatments.

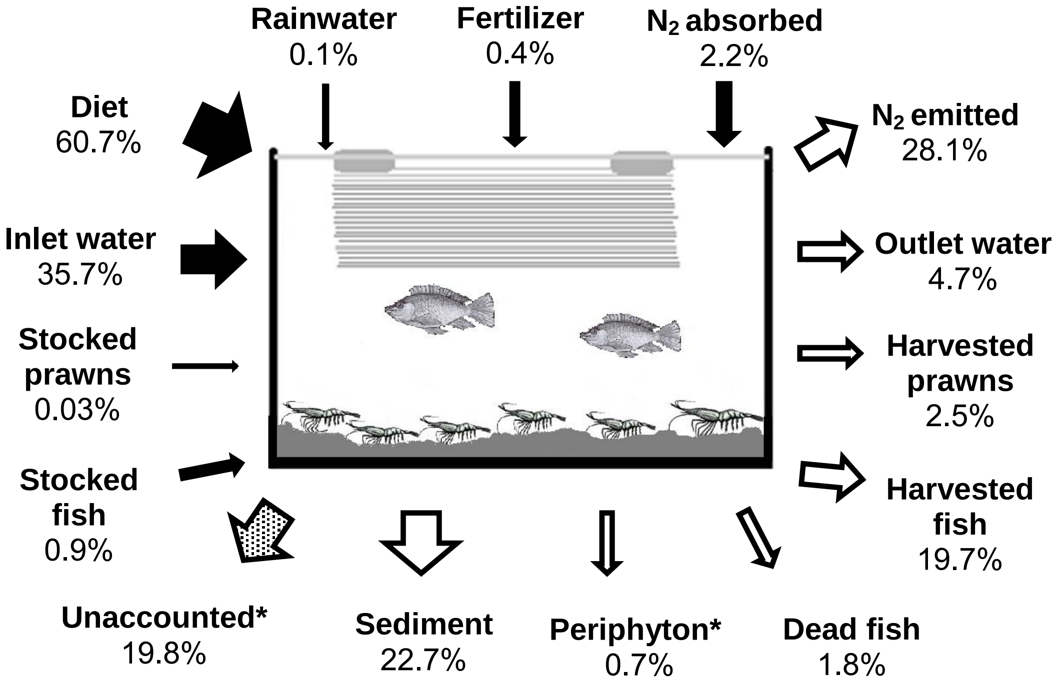

Figure 3. Nitrogen budget in Integrated Multi-Trophic Aquaculture system with *O. niloticus* and *M. amazonicum* in treatment with substrate made of bamboo (BS). Values are shown in percentages based on the total input to pond. *Indicate significant differences (P<0.05) between treatments.

4. Discussion

The addition of substrates had low effect on nitrogen budget of prawn-tilapia IMTA system. In general, no significant differences were observed in the content of nitrogen in each compartment and, thus, the nitrogen balance was similar in ponds with and without substrates. Furthermore, substrates did not improve the recovery or the accumulation of nitrogen in the biomass of reared animals. Less than 2.5% of added nitrogen was recovered by periphyton. These results indicate low productivity and turnover rate of periphyton or small substrate area available for colonization.

Most of the available nitrogen was not incorporated into fish or prawn biomass, but lost to other compartments. Nitrogen entered in the system mainly by diet supplied to tilapia (~61-71%) and inlet water (~26-36%). Most of this nitrogen was accumulated in pond bottom (~23-38%) or released to the atmosphere as N_2 (~28-36%). Thus, only ~19-20% of the total nitrogen input was retained in fish and ~2-3% in prawns. These values indicates that the system is inefficient in the use and retention of this key element, since more than 75% of available nitrogen is lost to environment.

Feed is the most representative input of nutrients in intensively fed systems. For instance, the addition of nitrogen in shrimp monocultures ranges from ~72 to 99% (Adhikari et al., 2014; Casillas-Hernandez et al., 2006; Sahu et al., 2013a; Saraswathy et al., 2013). The high contribution of inlet water to the total nitrogen input observed in the present work is the result of large volume of nutrient-rich water used throughout the culture to replenish evaporation and seepage (~9.5% daily). In previous studies, inlet water contributed with ~0.5 to 14% (Adhikari et al., 2014; Casillas-Hernandez et al., 2006; Sahu et al., 2013a; Saraswathy et al., 2013). The use of nutrient-rich water is a feasible alternative for aquaculture because has similar characteristics to the water found in aquaculture ponds and may represent a source of unpaid nutrients since it may be incorporated into reared animals (Kimpara et al., 2011).

Of the diet input, the portion of nitrogen recovered by fish was ~25 to 31%. It is expected that the addition of substrates improve the nitrogen retention in reared animals. This gain would be via periphyton food web. Tilapias graze more efficiently on the substrates rather than plankton diluted in the water column (Dempster et al., 1993) and prawns have the periphyton as an additional food source besides the wastes of tilapia culture. Nonetheless, the addition of substrates did not affect significantly the performance of fish and prawns to retain nitrogen. This suggests that the periphyton had a minor role on feed availability. Conversely, some studies have demonstrated that the addition of substrates in ponds increases animal biomass (Tidwell et al., 1999; Azim et al., 2001; Uddin et al., 2009; Haque et al., 2015). The production increase is proportional to the increase in the surface area of substrates (Tidwell et al., 2000; Tidwell et al., 2002). Perhaps, in the present work, some environmental factor may have reduced the total periphyton development or the substrate area was not enough to produce the amount of periphyton needed to feed the farmed animals.

Nitrogen retained in periphyton was low. This was 0.7 and 2.1% in treatments with substrate made of bamboo (BS) and geotextile blanket (GS), respectively. This result shows that geotextile blanket substrate retains more than double of nitrogen compared to bamboo. This difference, however, is related to periphyton dry mass (DM) adhered on different substrates per surface area (12.7 ± 5.7 g DM/m² for BS and 40.7 ± 13.8 g DM/m² for GS). The geotextile blanket substrate has an open structure (high porosity) that increases periphyton conditions to be adhered on substrate, which is not possible in bamboo. Nonetheless, retained nitrogen trapped within the pores of geotextile blanket substrate could not be grazed by the reared animals. Thus, no significant differences were found between periphyton-based treatments.

In monoculture ponds of freshwater fish, nitrogen recovery of total input varies from ~18 to 21% (Acosta-Nassar et al., 1994; Green and Boyd, 1995) and nitrogen recovery of diet input varies from ~21 to 27% (Boyd, 1985; Siddiqui and Al-Harbi, 1999). In monoculture ponds of prawns, nitrogen recovery of total input is reported to be ~37% (Sahu et al., 2013b; Adhikari et al., 2014). In the present work, the percentages of nitrogen recovery by fish was similar with the ranges reported in the literature. Nonetheless, the recovered portion by prawns was lower. This difference can be related with the amount of biomass produced and the type of feed. In monocultures, prawns are cultivated more intensively and fed with specific diets, which ensures a better performance in nitrogen retention. In this prawn-tilapia IMTA system, prawns were reared as co-cultured organisms in order to improve the nutrient loading of the system and explore the synergistic interactions between both species. Thus, the retained nitrogen in prawns biomass

(2 – 3%) is the result of nutrients reuse (feed conversion ratio is 0:1), which may be considered as a gain of nutrients to the system. It is known that nitrogen recovery by reared animals increases as the system is intensified (Sahu et al., 2013a) and that *M. amazonicum* tolerates grow-out intensification in monoculture ponds at least until 80 PL/m² (Moraes-Valenti and Valenti, 2007). Therefore, in future experiments, increased densities of prawns should be tested in this IMTA model in order to optimize even more the nutrient loading of the system.

There are several works showing that sediment is the major sink of nitrogen in aquaculture ponds, ranging from 29–47% in polycultures (Nhan et al., 2008; Sahu et al., 2015) and from 14–53% in monocultures (Thakur and Lin, 2003; Sahu et al., 2013b; Saraswathy et al., 2013). This accumulation is related to the buffering effect of sediment, which removes nutrients from the water and stores them (Chien and Lai, 1988). These nutrients are essentially organic and its main sources are uneaten feed, feces and dead plankton (Jimenez-Montealegre et al., 2005). In the present work, organic matter sedimentation was significantly lower in periphyton-based treatments and this result indicates that the addition of substrates in ponds changes water-sediment relationships. Periphyton is a complex mixture of autotrophic and heterotrophic organisms, which traps nutrients from the water column (Milstein et al., 2003) and, thus, reduces particle sedimentation (van Dam et al., 2002). This process occurs throughout the culture because grazing pressure of reared animals causes a rejuvenation of periphyton cells (Azim et al., 2003), avoiding thereby the excess of OM in the pond bottom. The biochemical conditions of the pond bottom are related to OM content, which in excess can diminish the metabolic capacity of the sediment (McKindsey et al., 2006) and favor anaerobic pathways (Kristensen and Holmer, 2001; Martinez-Garcia et al., 2015). Therefore, periphyton contributes to maintain the benthic ecosystem under low organic enrichment conditions and, consequently, favors aerobic metabolic pathways in substrate-based ponds. Another factor that avoided OM in excess was the simultaneous rearing of fish and prawns within the same aquaculture system. Even the higher OM concentration in the sediment of this prawn-tilapia IMTA system (WS treatment with ~43% of DM) was approximately half of the value reported by Kimpara et al. (2011) in monoculture of *M. amazonicum* (~84% of DM). This might be explained by the different cultivation technique and feed composition used in the respective cultures, which can influence OM concentration (Holmer et al., 2005; Stigebrandt et al., 2004). Kimpara et al. (2011) used pelleted diet for feeding prawns (which sinks and accumulates in the pond bottom) and the leftovers were not reused by another commercial species. In the present work, we used extruded diet (floating) for feeding tilapias and its leftovers were removed from the ponds. In addition, prawns consumed part of the waste deposited on the sediment. Thus, IMTA systems, even without substrates, generate less OM in the sediment than prawn monocultures and, consequently, enhance its metabolic capacity.

Nitrogen loss to the atmosphere as N_2 during the experiment (diffusion + bubbling) ranged from ~28–36% with no significant differences among treatments. In aquaculture systems, the loss of nitrogen through gaseous emissions is estimated around 20% of the nitrogen input (Hu et al., 2012). In the present work, however, a higher portion was lost to the atmosphere. This portion corresponds to the non-recovered nitrogen by target biomass, which after some transformation processes was emitted as $N_{2.}$ The presence of periphyton in the system could have changed this mechanism, since in substrate-based systems more nitrogen is entrapped in algal biomass (Kanan-Brou and Guiral, 1994) and in reared animals (Azim et al., 2003), making portions of nitrogen unavailable to denitrification process. Nonetheless, the apparent low periphyton biomass and turnover rate did not affect such mechanism and, thus, a high portion of nitrogen returned to the atmosphere. Of total N_2 loss, an average of 97.5% corresponds to the N_2 released by bubbles formed in the sediment at a rate ranging from ~112 to 123 $mg/m^2/d$. As this is a process influenced by many factors (oxygen, pH, temperature, nitrate concentration, organic carbon and population density of denitrifying bacteria), the emission products from different aquatic ecosystems can vary greatly, depending on the environmental conditions (Hargreaves, 1998; Hu et al., 2012). In the present work, the bubbling rate removed throughout the culture ~32% of non-recovered nitrogen. This result reinforces the important role of denitrification to buffer the impact of high loads of nutrients in ponds (Hargreaves, 1998) and the growing interest from aquaculture in processes that remove nitrogen from the water (Pretty et al., 2003).

Nitrogen released in outlet water during harvest ranged from ~5 to 6% and no differences were found among treatments. This result is probably related to the apparent low periphyton biomass, which did not entrap nutrients enough to decrease the amount of nitrogen in the outlet water. Similar percentages were found in cultures with no water exchange, ranging from ~3 to 8% (Adhikari et al., 2014; Sahu et al., 2013a; Sahu et al., 2013b). Nonetheless, cultures with water exchange showed higher percentages, ranging about ~16 to 34% (Casillas-Hernandez et al., 2006; Saraswathy et al., 2013). This suggests that closed systems are more efficient and environmentally friendly than continuous water flow systems. Nevertheless, as the outlet water remains nutrient-rich, some alternatives can be adopted to preserve the health of adjacent water bodies as the treatment of effluent with wetlands populated with aquatic macrophytes (Santos and Camargo, 2015) or direct discharging into rice fields and gardens (Phan et al., 2009).

Unaccounted nitrogen (total input less total output) ranged from ~0.4 to 20% and BS treatment had the highest portion. In BS and WS treatments, the inputs were greater than the outputs and, thus, we considered the unaccounted portions as nitrogen that came out of the system without being identified by analyzes. In case of GS treatment, it was the opposite. The unaccounted nitrogen can be related to some factors: loss of nitrogen compounds by seepage (Gross et al., 2000), the conversion of NO_3^- in N_2O (Hu et al., 2012) and NH_3 volatilization (Gross et al., 1999). Other factors may also have contributed to the unaccounted nitrogen as fixation by cyanobacteria; leaves that fell within the ponds; the fauna that developed in ponds, as small fish; migration of animals that can enter the system, deposit or consume nutrients, and leave the system; predation of fish and prawns, mainly by birds and even methodological errors. Most of the studies reported less than 20% of unaccounted nitrogen (Martin et al., 1998; Sahu et al., 2013a; Van Khoi and Fotedar, 2010, Sahu et al., 2015). Nonetheless, much higher losses from ~27 to 66 % have also been reported (Boyd, 1985; Paez-Osuna et al., 1999).

5. Conclusions

The mapping of nitrogen in each compartment of the system allowed the understanding of invested resources destination in this prawn-tilapia IMTA system. The addition of different types of substrates did not improve the recycling of nitrogen because the development of periphyton was low. Additional analyses like measuring animal grazing and satiation after feeding, productivity and turnover rates of plankton, benthos and periphyton communities could help in the understanding of system's efficiency path and, thus, should be performed in further studies. In general, the system is not efficient in the use of nitrogen since only about 20% of all available nitrogen were driven for prawns and fish biomass. Some alternatives could be tested to investigate the improvement of nitrogen recycling in this prawn-tilapia IMTA, such as the increase in prawns' stocking density, the addition of other detritivores-iliophagus (mud eating) species, the increase of substrates surface area and/or the reduction of the feed rate in order to force the animals to eat periphyton and wastes.

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CAPÍTULO 3

PHOSPHORUS BUDGET IN INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEMS WITH NILE TILAPIA AND AMAZON RIVER PRAWN

Phosphorus budget in integrated multi-trophic aquaculture systems with Nile Tilapia and Amazon river prawn

Abstract

The aim of the present study was to develop a phosphorus (P) budget analyzing the main compartments of Integrated Multi-Trophic Aquaculture (IMTA) systems of Amazon River prawn (Macrobrachium amazonicum) and Nile tilapia (Oreochromis niloticus), with and without the addition of different substrates. The experimental design was completely randomized, with three treatments and four replications: control, without substrate (WS); substrate made of geotextile blanket (GS); and substrate made of bamboo (BS). Nutrient budget revealed that feed was the major input of P (~52 to 60%) followed by the inlet water (~17 to 32%) and the fertilizer (~6 to 7%). The portion of P retained in reared animals and periphyton ranged from ~20 to 28% (being 18.3 to 24.6% in fish; 1.3 to 1.9% in prawns; 1.7 to 2.4% in periphyton). The P outputs occurred primarily by accumulation in the sediment (~57 to 68%) and by discharge of outlet water (~7 to 10%). The adopted IMTA system showed satisfactory results. Nonetheless, the addition of substrates did not affect significantly the performance of fish and prawns to retain P. This suggests that periphyton had low biomass and turnover rate and, thus, played a minor role on feed availability. The main advantage afforded by periphyton was the decrease of P concentration in water. Periphyton development needs further attention in future experiments. In addition, some possibilities can be tested to improve the use of stored P in sediment, such as increased density of prawns, the addition of other detritivore-iliophagus (mud eating) species and the reduction of the feed rate to force the animals to eat periphyton and wastes.

Introduction

Aquaculture activities involve a great variety of inputs in order to produce animal biomass, such as diet, fertilizer and water. Nonetheless, substantial part of the added nutrients is not accumulated in the biomass of reared animals, but ends up in the sediment and in the outlet water (Lin et al. 1997), which is a nutrient loss. To reduce this loss, a clear need exists to move toward Integrated Multi-Trophic Aquaculture systems (IMTA) as a key component for ensuring more efficient resource use and produce less waste (Chopin et al. 2001; Neori et al. 2004; Diana et al. 2013).

The IMTA system consists in simultaneous rearing of two or more aquatic species with complementary ecosystem functions (Chopin 2006). The combination of species in terms of ecosystemic functionalities is what differs IMTA from polyculture, where different species can be farmed together sharing the same general biological, physiological, nutritional and chemical processes (Chopin 2013). The IMTA system, thus, explores the synergistic interactions between species through the conversion of one species' uneaten feed and wastes, nutrients and by-products into fertilizer, feed, and energy for the other crops (Chopin 2006; Chopin et al. 2012). The Nile tilapia (Oreochromis niloticus) swims actively in the water column and can filter plankton, while the Amazon River prawn (Macrobrachium amazonicum) have benthonic habit and feed mainly on detritus and benthic organisms. These characteristics allow the exploitation of different niches in ponds and a better use of culture resources, since Nile tilapia may represent the fed organism and the Amazon River prawn the recycler organism.

In addition to the benefits of IMTA systems to feed optimization, it is possible to cultivate the animals with greater welfare (Tidwell et al. 1999; 2000) and regenerate the lost nutrients in periphyton (Saikia 2011) by adding substrates in the systems. The addition of substrates in ponds increases the useful area for benthic species, reducing agonistic encounters, and consequently, reducing stresses (Tidwell et al. 1999; 2000). Furthermore, it promotes an increase of space for colonization by periphyton, creating a new compartment that accumulates nutrients and makes them available for the reared species (Miller and Falace 2000; Saikia and Das 2015). Several studies have documented the benefits of adding artificial substrates to production units (Asaduzzaman et al. 2009; Azim et al. 2002; Uddin et al. 2008; Milstein et al. 2008). Nonetheless, the budget of nutrients was not evaluated.

The first step to the understanding of nutrient cycling in aquaculture systems is to quantify the key elements, such as phosphorus, in each compartment (Mariscal-Lagarda and Paez-Osuna 2014). The understanding of phosphorus budget has the potential of improving culture efficiency (Liu et al. 2014), providing waste reduction and enabling the management optimization of culture ponds (Adhikari et al. 2014). Thus, the objective of this work is to describe the phosphorus budget, by characterizing the inputs and outputs and quantifying the phosphorus content in all compartments of fish/prawn IMTA systems. In addition, we tested the hypothesis that the addition of substrates increases the retention of phosphorus according to the type of substrate.

Materials and Methods

The study was conducted in the Crustacean Sector of the Aquaculture Center, at São Paulo State University, Brazil (21°15’22’’S, 48°18’48’’W). We used twelve earthen ponds (oxisols) with ~0.01 ha and 1 m of water depth. The experimental design was completely randomized, with three treatments and four replications: (1) control, without substrate (WS); (2) substrate made of geotextile blanket (GS); and (3) substrate made of bamboo (BS).

Pond management

We used a system with no water exchange, adding inlet water only to compensate water lost by seepage and evaporation. This water receives high loads of nutrients from local fish farming and, thus, it is nutrient-rich. Except for the control group, each pond received substrates equivalent to 50% of its water surface area (Tidwell et al. 2004). The substrates were arranged vertically inside the ponds with the aid of floating plastic bottles. Additional substrates were installed inside net fences to avoid predation and were used for periphyton analysis. All ponds were fertilized with urea and simple superphosphate at the rate of 2 kg N/ha and 8 kg P_2O_5/ha, and then were left untouched for 10 days to allow plankton and periphyton growth. After this period, juveniles of M. amazonicum (0.03 ± 0.01 g) were stocked at a density of 21.5 individuals/m². After five weeks, juveniles of O. niloticus (29.0 ± 1.1g) were stocked at a density of 1.16 individuals/m^2.

The same feed regime was used in all ponds. Until stocking of tilapia, prawns were fed with pelleted diet (35% crude protein) at a rate of 10% of body weight two times a day. After that, prawns were not fed anymore. Tilapias were fed daily with a pelletized diet (40% crude protein in the first month and 28% for the rest of the culture period) at a rate of 4-2 % of tilapia biomass that was adjusted monthly. Daily feed was divided in two equal portions, at 12:00 and 16:00 hours. The amount of feed not consumed by tilapias 15 minutes after each feeding time (leftover) was removed from the ponds and discounted from values of supplied diet. Every month, 30 tilapias and 50 prawns were randomly sampled to take biometric measurements and to recalculate feed quantity. After the biometric analysis, the animals returned to the ponds.

Pond water quality

To characterize the culture, the following variables of water quality were measured biweekly: total ammonia nitrogen (APHA 2005; 4500-NH_3 F. Phenate method), nitrite nitrogen colorimetric (APHA 2005; 4500-NO_2^- B. Colorimetric method), nitrate nitrogen (APHA 2005; 4500-NO_3^- E. Cadmium reduction method), transparency (Boyd 1979; Secchi disc) and total suspended solids (APHA 2005; TSS dried at 103-105ºC). Temperature and dissolved oxygen (DO) were monitored daily and pH was measured weekly. These parameters were determined in situ (at 20-30 cm below the water surface) at 08:00 hours using a digital YSI professional plus (Yellow Springs Instruments, Yellow Springs, OH, USA). Water quality parameters are given in Table 1. Emergency aerators were used when DO declined bellow 1.5 mg/L.

Table 1. Means (±SD) of water quality parameters in IMTA¹ system with O. niloticus and M. amazonicum obtained from the following treatments: without substrate (WS), geotextile substrate (GS) and bamboo substrate (BS). Minimum and maximum values reached are shown inside parentheses.

Water quality parameters	**Treatments**		
	WS	**GS**	**BS**
T (ºC)	27.1 ± 0.9 (20.5 – 29.4)	27.1 ± 0.9 (20.5 – 29.3)	27.1 ± 0.9 (22.7 – 29.5)
DO (mg/L)	4.5 ± 1.3 (0.8 – 9.4)	4.0 ± 1.5 (0.8 – 9.2)	4.1 ± 1.2 (0.8 – 9.0)
pH	7.87 ± 0.45 (7.18 – 9.13)	7.88 ± 0.15 (7.21 – 8.79)	7.71 ± 0.20 (7.14 – 8.25)
$N\text{-}NH_3$ (µg/L)	138.4 ± 35.2 (16.6 – 561.1)	143.5 ± 30.5 (25.5 – 465.2)	109.3 ± 23.6 (6.8 – 303.7)
$N\text{-}NO_2^-$ (µg/L)	8.1 ± 3.6 (0.2 – 69.4)	11.2 ± 3.2 (0.6 – 70.7)	5.8 ± 1.6 (0.4 – 21.1)
$N\text{-}NO_3^-$ (µg/L)	53.0 ± 27.0 (1.8 – 241.8)	85.6 ± 25.3 (1.5 – 270.3)	43.2 ± 24.5 (1.4 – 168.9)
Transparency (cm)	34.6 ± 2.1 (8 - 74)	39.5 ± 4.7 (13 - 82)	35.0 ± 4.7 (13 - 74)
TSS (mg/L)	32.0 ± 23.1 (8.1 – 85.1)	32.2 ± 24.6 (9.3 – 76.9)	29.7 ± 19.1 (9.7 – 57.4)

¹ IMTA: Integrated Multi-trophic Aquaculture.

Harvest

The experiment ended on the 140th day because of the cold weather (mid-autumn). All ponds were dried, totally harvested and all animals were counted. To determine individual weight, we analyzed all fish and a random sample of 10% of all prawns from each pond. The animals were weighted (Precision Balance Mate-AS2000C; 0.1 g precision) and measured (wood caliper; 1 mm precision).

Survival, mean weight and productivity were determined. These production variables are given in Table 2.

Table 2. Means (±SD) of production variables in IMTA¹ system with O. niloticus and M. amazonicum obtained from the following treatments: without substrate (WS), geotextile substrate (GS) and bamboo substrate (BS). Different letters in the same line indicate significant differences between treatments (*P*<0.05).

Production variables	**Treatments**		
	WS	**GS**	**BS**
O. niloticus			
Survival (%)	79.3 ± 7.4	86.7 ± 1.2	88.0 ± 3.2
Mean wet weight (g)	521.7 ± 42.8	493.2 ± 37.8	474.7 ± 58.5
Productivity (kg/ha)	4,794.4 ± 195.6	4,988.3 ± 404.3	4,852.6 ± 460.7
M. amazonicum			
Survival (%)	76.4 ± 4.4	64.5 ± 17.0	74.0 ± 9.3
Mean wet weight (g)	2.7 ± 0.2^{b}	3.5 ± 0.4^{a}	3.1 ± 0.5^{ab}
Productivity (kg/ha)	435.8 ± 15.4	483.1 ± 115.3	480.8 ± 29.1

¹ IMTA: Integrated Multi-trophic Aquaculture.

Nutrient budget

To calculate phosphorus budget we divided the system in compartments of input (inlet water, rainwater, fertilizer, stocked fish, stocked prawns and diet) and output (outlet water, harvested fish, harvested prawns, dead fish, periphyton and sediment). Subtracting the total input from the total output, we determined the unaccounted portion of phosphorus.

Phosphorus (P) input and output in water was calculated by multiplying total P concentration by total water volume. The concentration of P in the inlet water is related to the day of fish stocking (beginning of IMTA system) and in the outlet water to the day before harvest. To determinate the total P contained in water, the samples were submitted to a previous digestion, according to persulfate method (APHA 2005; 4500-P B.5.), to release the compounds associated with organic matter in the form of orthophosphate. Then, orthophosphate was measured by the Stannous Chloride method (APHA 2005; 4500-P D.). The inlet water volume is the total amount of water used to fill the ponds plus the volume lost by evaporation and seepage. Rainwater samples were collected five times during the experiment. We analyzed all samples and used a mean value of total P concentration. Rainwater volume was calculated using rainfall data from the Agrometeorological Station of UNESP Jaboticabal, Brazil. Total precipitation volume in the culture period (measured in L/m^2) was adjusted for each pond area, and then, multiplied by the mean P concentration in rainwater. For chemical fertilization, we used the P concentration provided by the manufacturer.

Samples of stocking and harvesting animals were analyzed in triplicate (AOAC 1995- 969.31 A) and the mean of total P concentration was multiplied by the total biomass of animals. All dead fish were removed from ponds throughout the experiment. Therefore, it was regarded as an outlet compartment. The total mass of dead fish in each pond was multiplied by the total P content retained in fish. The input of P through feed was calculated by multiplying total P concentration measured in diet (AOAC 1995- 969.31 A) by the total amount of diet supplied.

Retained P in periphyton was analyzed by samples of 10 cm width by 20 cm height collected from the additional substrates inside the net fences (between 20 and 40 cm from the water surface). We extracted the periphyton from the substrates using an ultrasonic homogenizer (USC – 750, Unique Group, São Paulo, SP, Brazil) according to Thompson et al. (2002) and analyzed the dry mass (AOAC 1995- 934.01) and the total P content (APHA 2005; 4500-P B.5.; 4500-P D.). From the dry mass sample, we estimated the entire mass adhered on the substrates. Then, to calculate the retained P in the substrates, we multiplied the entire periphyton mass by total P content.

Sediment samples were collected by tripton samplers (six 1.876 L PVC tubes, with 9.7 cm diameter and 25.4 cm long to 15 cm depth). One tripton sampler was placed on the bottom of each pond for 48 h biweekly. Samples were dried (AOAC 1995- 934.01) and analyzed to determine the P content (Michelsen 1957). From the total amount of sediment sampled in 48h considering the area of the tripton's sampler (~0.045 m²), we estimated the total amount of sediment accumulated in the pond. Then, to calculate the total P in sediment, we multiplied the accumulated mass by P content in the sample. Organic matter was determined by incinerating samples in a muffle furnace at 550º C (AOAC 1995 - 920.153).

Data analyses

Survival data was square root arcsine transformed prior to analysis. All data were tested for normality (Shapiro–Wilk) and homoscedasticity (Levene). As both conditions were satisfied, analysis of variance (One-Way ANOVA) was applied to verify the differences in variables of water quality, productions, and compartments of phosphorus budget. Productivity data of prawns were not normal and, therefore, were analyzed with Kruskal-Wallis test. Statistical analyses were carried out in R software version 0.98.945 (R Foundation for Statistical Computing, Vienna, Austria) and the level of significance considered was α = 0.05. When significant differences were detected among treatments, means were compared by Tukey's test.

Results

Total phosphorus loads and the comparisons among treatments are shown in Table 3. Total phosphorus percentages are represented as flow diagrams in Figures 1, 2, and 3. Total output values were higher than the input in treatments without substrate (WS) and substrate made of geotextile blanket (GS). In case of substrate made of bamboo (BS), the unaccounted phosphorus was identified in the input. Diet was the major input of phosphorus in all treatments, ranging from ~52 to 60%. The second most representative input was the inlet water, ranging from ~17 to 32%, and then, the fertilizer with a contribution ranging from ~6 to 7%. The other input compartments together ranged from 1.5 to 1.8%. No statistical differences were found among the treatments in the input compartments.

Phosphorus output occurred primarily by accumulation on the sediment, ranging from ~57 to 68%. The organic matter (OM) accumulated in the sediment accounted for ~34 to 43% of the dry matter and was significantly lower in periphyton-based treatments. Phosphorus retained (recovered) in reared animals and periphyton ranged from ~20 to 28% (being 18.3 to 24.6% in fish; 1.3 to 1.9% in prawns; 1.7 to 2.4% in periphyton). Phosphorus outputs in the outlet water ranged from ~7 to 10% and in dead fish ranged from ~2 to 3%. Only outlet water significantly differed among treatments; WS treatment discharges more P to receiving water bodies. The final budget showed that the unaccounted P ranged from ~5 to 24%; it was significantly higher in BS and lower in WS; both treatments did not differ from GS treatment.

Table 3. Means (±SD) of phosphorus budget in IMTA[1] system with O. niloticus and M. amazonicum obtained from the following treatments: without substrate (WS), geotextile substrate (GS) and bamboo substrate (BS). Different letters in the same line indicate significant differences between treatments (*P*<0.05).

Compartments (kg/ha)	**Treatments**		
	WS	**GS**	**BS**
Input			
Diet	73.1 ± 7.2	70.7 ± 4.1	68.6 ± 6.1
Inlet water	24.2 ± 3.6	28.8 ± 9.3	36.4 ± 12.6
Rainwater	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
Fertilizer	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0
Stocked fish	1.6 ± 0.1	1.6 ± 0.3	1.6 ± 0.1
Stocked prawns	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Output			
Outlet water	13.8 ± 3.3^{a}	8.4 ± 2.0^{b}	8.8 ± 1.5^{b}
Periphyton	-	3.1 ± 0.9	2.0 ± 0.7
Harvested fish	25.8 ± 3.0	26.0 ± 4.0	28.3 ± 4.0
Harvested prawns	1.8 ± 0.5	1.9 ± 0.5	2.2 ± 0.1
Dead fish	4.3 ± 1.5	2.7 ± 0.4	2.5 ± 0.3
Sediment	95.4 ± 29.4	86.4 ± 20.4	65.9 ± 20.2
Unaccounted			
Input - Output	-33.7 ± 22.2^{b}	-18.9 ± 16.2^{ab}	5.4 ± 15.3^{a}

[1] IMTA: Integrated Multi-trophic Aquaculture.

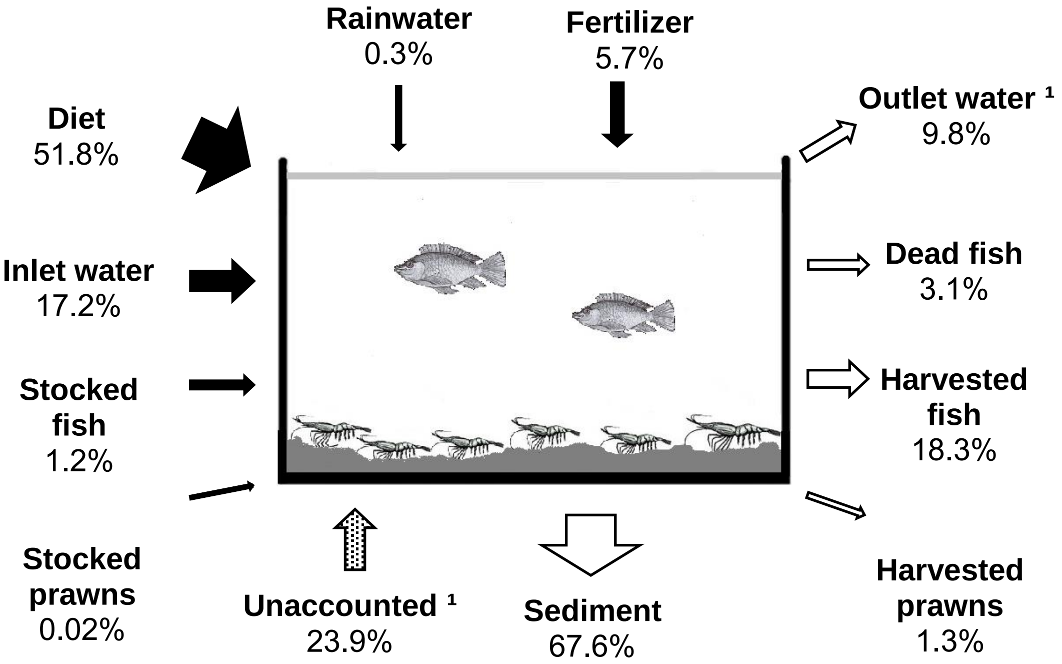

Figure 1. Phosphorus budget in integrated multi-trophic aquaculture system with O. niloticus and M. amazonicum in treatment without substrate (WS). Values are shown in percentages based on the total output to pond. ¹ Indicate significant differences (P<0.05) among treatments.

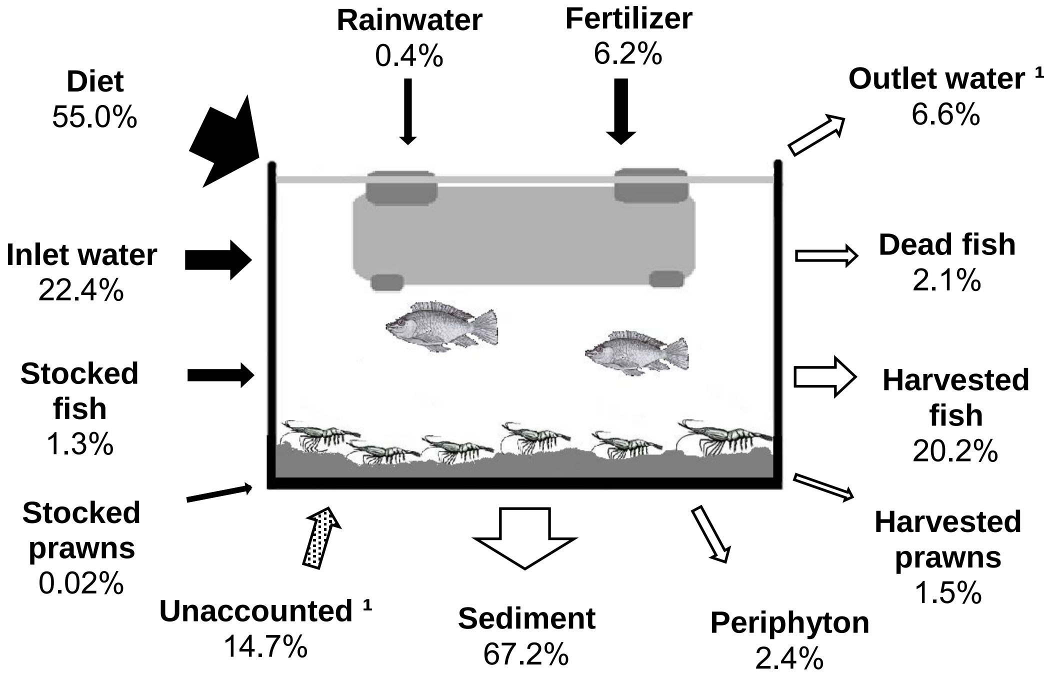

Figure 2. Phosphorus budget in integrated multi-trophic aquaculture system with O. niloticus and M. amazonicum in treatment with substrate made of geotextile blanket (GS). Values are shown in percentages based on the total output to pond. ¹ Indicate significant differences (P<0.05) among treatments.

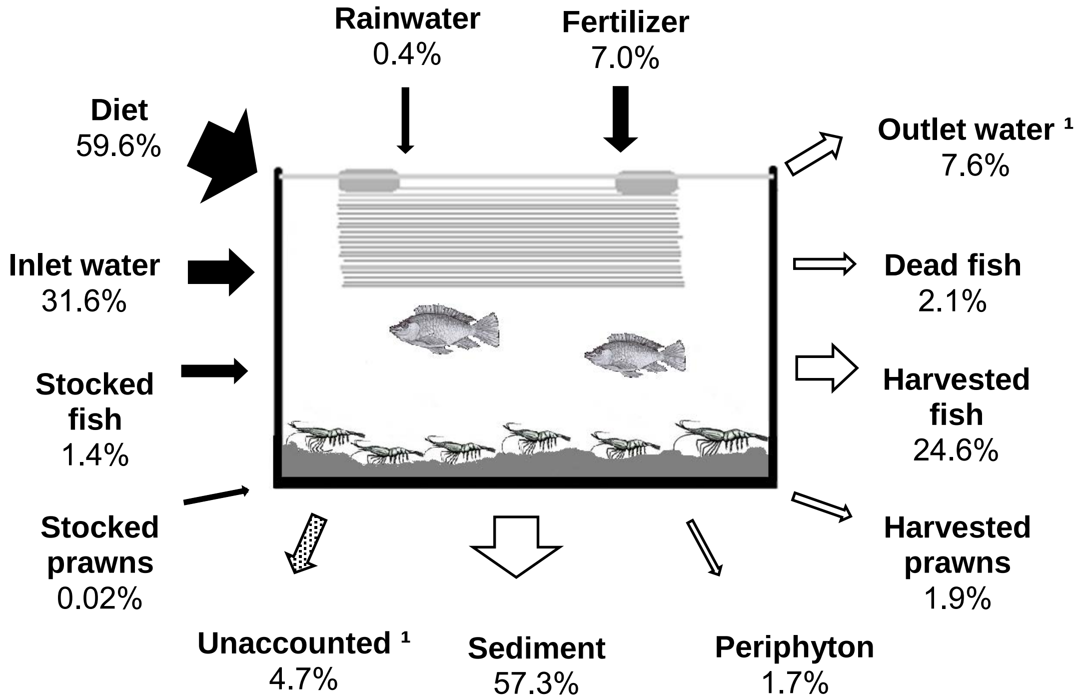

Figure 3. Phosphorus budget in integrated multi-trophic aquaculture system with O. niloticus and M. amazonicum in treatment with substrate made of bamboo (BS). Values are shown in percentages based on the total input to pond. ¹ Indicate significant differences ($P<0.05$) among treatments.

Discussion

Diet was the bulk of phosphorus inputs in this prawn/fish IMTA system (~52 to 60%). This result is normally observed in monocultures and indicates that the adopted IMTA system remains very dependent of external inputs of nutrients. Nevertheless, significant amount of prawns were produced with no specific addition of diet and consequently of phosphorus. Feed inputs were the major source of phosphorus in monocultures of Macrobrachium rosenbergii, ranging from ~98 to 99% (Sahu et al. 2013b; Adhikari et al. 2014) and in monocultures of Penaeus monodon, ranging from ~70 to 95% (Sahu et al. 2013a; Thakur and Lin 2003).

The inlet water had the second highest contribution on the total inputs of phosphorus (~17 to 32%), even with no water exchange. It has been observed that the contribution of inlet water varies largely, from 0.3% (Sahu et al. 2013b; Adhikari et al. 2014) to 38% (Casillas-Hernandez et al. 2006). This variation occurs because the quality and the volume of inlet water used to replace seepage and evaporation differ according to the local characteristics of each study. In the present study, the high contribution is because of the use of nutrient-rich water from a reservoir that receives aquaculture effluents, combined with the large volume used to replace loss by seepage and evaporation, which was ~9.5% daily. The reuse of anthropogenic nutrient loads is a recycling path, which ensures an optimization of available resources instead of adding nutrients periodically by fertilizing. This may represent a source of unpaid nutrients that can be incorporated into reared animals biomass (Kimpara et al. 2011), decreasing the load of nutrients in the water and improving systems efficiencies. Improvements in the design of the adopted IMTA system should be investigated to take advantage of this important source nutrients, which could decrease the input of phosphorus by commercial feed.

The retained portion of phosphorus in reared animals was lower than 30%. Even so, this prawn/fish IMTA system showed satisfactory results, since the retained portion by tilapia (~18 to 25%) was higher than the range reported in monocultures (10 to 20%) (Siddiqui and Al-Harbi 1999; Green and Boyd 1995). In addition, we had an extra portion retained in prawns (mean of 1.9%), which is a gain of nutrients to the system. Phosphorus retention in monocultures of P. monodon was reported to be from ~10 to 13% (Sahu et al. 2013a) and of M. rosenbergii around 10% (Adhikari et al. 2014). In the present study, the lower performance of prawns to retain phosphorus can be related with the type of feed and low prawn biomass. In monocultures, prawns are fed with specific diets, which ensure a better performance. In this IMTA system, prawns reused nutrients that were available in periphyton and waste products, which may have proportionated lower growth and, consequently, lower phosphorus retention. Moraes-Valenti and Valenti (2007) showed that M. amazonicum tolerates the increased stocking density in the grow-out phase at least until 80 PL/m² and Sahu et al. (2013a) showed that nutrient recovery increases with the intensification of the system. Therefore, in order to increase the phosphorus retention by prawns and better use the system resources, this IMTA model should be tested with increased density of prawns.

Retained phosphorus in periphyton was low (1.7 to 2.4%) and did not differ between treatments. These data, however, represent only the phosphorus retained in periphyton at harvest and, probably, do not represent the entire assimilation during the culture. Fluctuations of periphyton population occur throughout the culture because of the processes of ecological succession and detachment on substrates (Saikia 2011). Nevertheless, even considering the phosphorus retention by periphyton during the culture, it did not improve the conversion of this nutrient in prawn/fish biomass as expected. This result indicates low productivity and turnover rate of periphyton or small substrate area available for colonization. On the other hand, periphyton was able to retain phosphorus from the water, as observed in lakes (Pei et al. 2015), and it brought benefits to the system such as maintenance of pond water quality (Hogan et al. 2004; Milstein et al. 2005).

Released phosphorus in the outlet water (~7 – 10%) was significantly lower in treatments with addition of substrates, supporting the idea that periphyton sink phosphorus and, thus, periphyton-based cultures cause less impact to the receiving water bodies at harvest (Azim et al. 2003; Milstein et al. 2003; Gaiser et al. 2004). Culture in stagnant ponds tend to release lower phosphorus in the outlet water and accumulate it in the sediment (Sahu et al. 2013b; Adhikari et al. 2014), when compared to systems with water exchange (Casillas-Hernandez et al. 2006). This is because of the increased water residence period and, consequently, increased sedimentation rate (Saunders and Kalff 2001). The outlet water is generally discharged in the surrounding environment; thus, it is preferable that residual phosphorus ends up in the sediment to avoid eutrophication in receiving waters.

Sediment is commonly the main output of phosphorus in aquaculture systems. This compartment accumulated ~57 to 68% of total phosphorus in the adopted IMTA system, ~43 to 76% in prawn monocultures (Casillas-Hernandez et al. 2006; Adhikari et al. 2014; Sahu et al. 2013b) and about 51% in fish polyculture (Nhan et al. 2008). The addition of substrates did not affect phosphorus accumulation on sediment, since no significant differences were found among treatments. Nonetheless, in periphyton-based treatments, the organic matter sedimentation was significantly lower. This element is incorporated into sediments in both inorganic and organic forms and some fractions can be released to the overlying water, depending on pH, redox potential, environmental conditions, etc. (Di Luca et al. 2015). Thus, sediment can act as a sink or/and source of phosphorus to the culture and plays an important role in buffering the water nutrient concentrations (Chien and Lai 1988). The net flux of phosphorus from the water into the sediment, however, is higher. This is because sediment has a strong affinity for phosphorus (Shrestha and Lin 1996) and, generally, sediment layers of few centimeters depth contains more nutrients than the whole water column (Avnimelech et al. 1984). Thus, sediment may be removed from aquaculture ponds and used to fertilize vegetable cultures. This practice may be an important way to increase the use of the phosphorus available in aquaculture ponds. Studies to assess the technical and economic feasibility of this procedure should be performed.

Unidentified portions are common in mass balances analyses. Several studies indicate rates ranging from ~12 to 34% (Le Van and Fotedar 2011; Van Khoi and Fotedar 2010; Adhikari et al. 2014; Sahu et al. 2013a; Sahu et al.

2013b). Generally, estimated inputs are greater than outputs as observed in the present work for ponds provided with bamboo substrates (mean of 4.7%). Conversely, the estimated phosphorus outputs surpass inputs in 14.7% and 23.9% (means of each treatment) in WS and GS treatments, respectively. Unaccounted phosphorus may be lost by seepage, incorporated in small fish (not computed), entered in ponds as fallen plant leaves, dust, feces of birds or leave the ponds by migration of animals that can enter in the system, deposit or consume nutrients, and leave the system, predation of fish and prawns by terrestrial animals and even caused by methodological errors.

We have characterized the main attributes of the phosphorus budget of prawn/fish IMTA systems with and without addition of substrates, which can support future efforts toward improve conversion of inputs in harvestable products. The addition of different types of substrates in the system did not improve the performance of fish and prawns to retain phosphorus. This suggests that periphyton had low biomass and turnover rate, playing a minor role on feed availability. The main advantage afforded by periphyton was the decrease of P concentration in water, resulting lower loads in the effluents and, consequently, less impact to receiving water bodies. This approach showed a promising start that can be improved with some adaptations and further attention on periphyton development. Some possibilities can be tested in future research to improve the use of stored phosphorus in sediment, such as increased density of prawns, the addition of other detritivore-iliophagus (mud eating) species and the reduction of the feed rate to force the animals to eat periphyton and wastes.

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CAPÍTULO 4

CARBON BUDGET IN INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEMS WITH NILE TILAPIA AND AMAZON RIVER PRAWN

Carbon budget in integrated multi-trophic aquaculture systems with Nile tilapia and Amazon river prawn

Abstract

The aim of the present study was to investigate the use of carbon (C) in Integrated Multi-Trophic Aquaculture (IMTA) systems with Nile tilapia (*Oreochromis niloticus*) and Amazon River prawn (*Macrobrachium amazonicum*), with and without the addition of different substrates. The experimental design was completely randomized, with three treatments and four replications: control, without substrate (WS); substrate made of geotextile blanket (GS); and substrate made of bamboo (BS). Nutrient budget revealed that the major input of nutrient was the diet (~54 to 63% Organic C) followed by the inlet water (~29 to 39% Total C) and absorbed gases (~5 to 7% C). The retained portion in reared animals and periphyton ranged from ~13 to 14% OC (being 10.9 to 12.0% in fish; 1.0 to 1.2% in prawns; 1.2% in periphyton). Carbon outputs occurred primarily by accumulation in the sediment (~38 to 70% OC), by discharge of the outlet water (~12 to 13% TC) and by gas emission (~2 to 3% C). The characterization of the carbon budget allowed the understanding of invested resources destination and showed that the addition of substrates in the adopted IMTA system promoted different OC retentions in fish tissues, but with no improvement on system efficiency. Further attention on periphyton development has to be considered in future researches in order to understand the lack of treatment effects on system efficiency. Atmospheric carbon sequestration in IMTA systems shows a positive externality of aquaculture. Some possibilities can be tested to improve the results of this approach, such as increase the stocking density of prawns, reduce the feed rate to force the animals to eat periphyton and wastes, add other detritivore-iliophagus (mud eating) species in the system and use sediment as fertilizer.

Introduction

Aquaculture has undergone a transition process toward an ecological approach which aims improve not only the technical aspects of biomass cultivation but also the management of natural resources (Costa-Pierce, 2010). Such transition attempts to mitigate ecological problems caused by aquaculture facilities and contributes for ensuring long-term sustainability in the aquaculture industry (Chopin et al., 2001; Neori et al., 2004; Brown, 2009). An aquaculture technology that is becoming relevant in this field of study is the IMTA (Integrated Multi-Trophic Aquaculture) system, in which species from different trophic niches and with complementary ecosystem functions grows in the same installation (Chopin et al., 2012), providing better use of available resources. Along with increasing system's efficiencies, the IMTA systems improve ecosystem health, offer diversified products to the market, have societal acceptability, and increase farms' biosecurity (Crab et al., 2007; Martinez-Espineira et al., 2015; Pietrak et al., 2012).

The IMTA concept is flexible and can be applied to different approaches, such as marine or freshwater systems and temperate or tropical systems (Chopin et al., 2012). The main characteristic of this technology is the connectivity of reared species in terms of ecosystemic functionalities (Barrington et al., 2009). The Nile tilapia (*Oreochromis niloticus*) and the Amazon river prawn (*Macrobrachium amazonicum*) inhabit different niches in ponds, since tilapia swims actively in the water column and filters plankton whilst prawns have benthic habit and feed mainly on detritus and benthic organisms. This combination allows the exploitation of both niches and reduces waste, since uneaten feed and waste products from one species are used to feeding the other (Chopin et al., 2001).

Beyond the benefits of IMTA approach, system's efficiencies can still be optimized by the addition of substrates. Layers of horizontal surfaces inside the ponds increases useful area and minimizes territorial effects (Tidwell and Bratvold, 2005), improving the welfare of benthic species (Tidwell et al., 2000). Furthermore, promotes an increase of space for periphyton settlement, which regenerates the lost nutrients in an additional natural food for reared species (Milstein et al., 2003; Haque et al., 2015). Several studies have documented the advantages of adding artificial substrates to aquaculture systems (Milstein et al., 2008; Uddin et al., 2008; Asaduzzaman et al., 2009). Nonetheless, these studies do not include nutrient budgets, which are important to understand the efficiency of input resources.

The key of aquaculture development is the maximization of available nutrients, which requires detailed understanding of the most important biogeochemical cycles and their interactions. This overall view of nutrients in ponds may occur through mass budgets, which quantifies the key elements in each compartment of the systems. This can help identify the efficiency of input resources and the feasibility of practices that have potential to make aquaculture more sustainable and profitable. A quantitative study on carbon budget is a prerequisite to understand the emissions and sequestrations (Adhikari et al., 2012) and the metabolism (Coletti et al., 2013) of this element in aquaculture ponds. Thus, the objective of this work is to describe the carbon budget, by characterizing the inputs and outputs and quantifying the carbon content in all compartments of fish/prawn IMTA systems. In addition, we tested the hypothesis that the addition of substrates increases the retention of carbon according to the type of substrate.

Materials and Methods

The study was conducted in the Crustacean Sector of the Aquaculture Center, at São Paulo State University, Brazil (21°15’22’’S, 48°18’48’’W). We used twelve earthen ponds (oxisols) with ~0.01 ha and 1 m of water depth. The experimental design was completely randomized, with three treatments and four replications: (1) control, without substrate (WS); (2) substrate made of geotextile blanket (GS); and (3) substrate made of bamboo (BS).

Pond management

We used a system with no water exchange, adding inlet water only to compensate water lost by seepage and evaporation. This water receives high loads of nutrients from local fish farming and, thus, it is nutrient-rich. Except for the control group, each pond received substrates equivalent to 50% of its water surface area (Tidwell et al., 2004). The substrates were arranged vertically inside the ponds with the aid of floating plastic bottles. Additional substrates were installed inside net fences to avoid predation and were used for periphyton analysis. All ponds were fertilized with urea and simple superphosphate at the rate of 2 kg N/ha and 8 kg P_2O_5/ha, and then were left untouched for 10 days to allow plankton and periphyton growth. After this period, juveniles of *M. amazonicum* (0.03 ± 0.01 g) were stocked at a density of 21.5 individuals/m^2. After five weeks, juveniles of *O. niloticus* (29.0 ± 1.1g) were stocked at a density of 1.16 individuals/m^2.

The same feed regime was used in all ponds. Until stocking of tilapia, prawns were fed with pelleted diet (35% crude protein) at a rate of 10% of body weight two times a day. After that, prawns were not fed anymore. Tilapias were fed daily with a pelletized diet (40% crude protein in the first month and 28% for the rest of the culture period) at a rate of 4-2 % of tilapia biomass that was adjusted monthly. Daily feed was divided in two equal portions, at 12:00 and 16:00 hours. The amount of feed not consumed by tilapias 15 minutes after each feeding time (leftover) was removed from the ponds and discounted from values of supplied diet. Every month, 30 tilapias and 50 prawns were randomly sampled to take biometric measurements and to recalculate feed quantity. After the biometric analysis, the animals returned to the ponds.

Pond water quality

To characterize the culture, the following variables of water quality were measured biweekly: total ammonia nitrogen (APHA, 2005; 4500-NH_3 F. Phenate method), nitrite nitrogen colorimetric (APHA, 2005; 4500-NO_2^- B. Colorimetric method), nitrate nitrogen (APHA, 2005; 4500-NO_3^- E. Cadmium reduction method), transparency (Boyd, 1979; Secchi disc) and total suspended solids (APHA, 2005; TSS dried at 103-105ºC). Temperature and dissolved oxygen (DO) were monitored daily and pH was measured weekly. These parameters were determined in situ (at 20-30 cm below the water surface) at 08:00 hours using a digital YSI professional plus (Yellow Springs Instruments, Yellow Springs, OH, USA). Water quality parameters are given in Table 1. Emergency aerators were used when DO declined bellow 1.5 mg/L.

Table 1. Means (±SD) of water quality parameters in IMTA* system with *O. niloticus* and *M. amazonicum* obtained from the following treatments: without substrate (WS), geotextile substrate (GS) and bamboo substrate (BS). Minimum and maximum values reached are shown inside parentheses.

Water quality parameters	Treatments		
	WS	**GS**	**BS**
T (ºC)	27.1 ± 0.9 (20.5 – 29.4)	27.1 ± 0.9 (20.5 – 29.3)	27.1 ± 0.9 (22.7 – 29.5)
DO (mg/L)	4.5 ± 1.3 (0.8 – 9.4)	4.0 ± 1.5 (0.8 – 9.2)	4.1 ± 1.2 (0.8 – 9.0)
pH	7.87 ± 0.45 (7.18 – 9.13)	7.88 ± 0.15 (7.21 – 8.79)	7.71 ± 0.20 (7.14 – 8.25)
N-NH_3 (µg/L)	138.4 ± 35.2	143.5 ± 30.5	109.3 ± 23.6

	(16.6 – 561.1)	(25.5 – 465.2)	(6.8 – 303.7)
$N\text{-}NO_2^-$ (µg/L)	8.1 ± 3.6 (0.2 – 69.4)	11.2 ± 3.2 (0.6 – 70.7)	5.8 ± 1.6 (0.4 – 21.1)
$N\text{-}NO_3^-$ (µg/L)	53.0 ± 27.0 (1.8 – 241.8)	85.6 ± 25.3 (1.5 – 270.3)	43.2 ± 24.5 (1.4 – 168.9)
Transparency (cm)	34.6 ± 2.1 (8 - 74)	39.5 ± 4.7 (13 - 82)	35.0 ± 4.7 (13 - 74)
TSS (mg/L)	32.0 ± 23.1 (8.1 – 85.1)	32.2 ± 24.6 (9.3 – 76.9)	29.7 ± 19.1 (9.7 – 57.4)

*IMTA: Integrated Multi-trophic Aquaculture.

Harvest

The experiment ended on the 140th day because of the cold weather (mid-autumn). All ponds were dried, totally harvested and all animals were counted. To determine individual weight, we analyzed all fish and a random sample of 10% of all prawns from each pond. The animals were weighted (Precision Balance Mate-AS2000C; 0.1 g precision) and measured (wood caliper; 1 mm precision). Survival, mean weight and productivity were determined. These production variables are given in Table 2.

Table 2. Means (±SD) of production variables in IMTA* system with *O. niloticus* and *M. amazonicum* obtained from the following treatments: without substrate (WS), geotextile substrate (GS) and bamboo substrate (BS). Different letters in the same line indicate significant differences between treatments (*P*<0.05).

Production variables	**Treatments**		
	WS	**GS**	**BS**
O. niloticus			
Survival (%)	79.3 ± 7.4	86.7 ± 1.2	88.0 ± 3.2
Mean wet weight (g)	521.7 ± 42.8	493.2 ± 37.8	474.7 ± 58.5
Productivity (kg/ha)	4,794.4 ± 195.6	4,988.3 ± 404.3	4,852.6 ± 460.7
M. amazonicum			
Survival (%)	76.4 ± 4.4	64.5 ± 17.0	74.0 ± 9.3

Mean wet weight (g)	2.7 ± 0.2^{b}	3.5 ± 0.4^{a}	3.1 ± 0.5^{ab}
Productivity (kg/ha)	435.8 ± 15.4	483.1 ± 115.3	480.8 ± 29.1

*IMTA: Integrated Multi-trophic Aquaculture.

Carbon budget

To calculate carbon budget we divided the system in compartments of input (inlet water, rainwater, stocked fish, stocked prawns, diet and absorbed gases) and output (outlet water, harvested fish, harvested prawns, dead fish, periphyton, sediment and emitted gases). Subtracting the total input from the total output, we determined the unaccounted portion of carbon.

Carbon (C) input and output in water was calculated by multiplying total C concentration by total water volume. The concentration of C in the inlet water is related to the day of fish stocking (beginning of IMTA system) and in the outlet water to the day before harvest. To determinate the total C contained in water, the samples were analyzed by oxidation catalytic combustion (Shimadzu TOC-VCP).

The inlet water volume is the total amount of water used to fill the ponds plus the volume lost by evaporation and seepage. Rainwater samples were collected five times during the experiment. We analyzed all samples and used a mean value of C concentration. Rainwater volume was calculated using rainfall data from the Agrometeorological Station of UNESP Jaboticabal, Brazil. Total precipitation volume in the culture period (measured in L/m^2) was adjusted for each pond area, and then, multiplied by the mean C concentration in rainwater.

Samples of stocking and harvesting animals were analyzed in triplicate (AOAC, 1995- 920.153) and the mean C concentration was multiplied by the total biomass of animals. All dead fish were removed from ponds throughout the experiment. Therefore, it was regarded as an outlet compartment. The total mass of dead fish in each pond was multiplied by the C content retained in fish. The input of C through feed was calculated by multiplying C concentration in diet (AOAC, 1995- 920.153) by the total amount of diet supplied.

Carbon in form of gas (CO_2 and CH_4) was estimated by two analyses: diffusive and bubbling (Matvienko et al., 2001). For the first, we evaluated the diffusion at the air-water interface by the balance method with the aid of a diffusion chamber. This methodology allows a partial equilibrium between the gas dissolved in the water and the gas inside the chamber through the diffusion of gas to the water (absorption) or from the water (emission). Thus, a diffusion chamber was placed in contact with the surface of the water and one liter of air, collected as close as possible to the air-water interface, was placed inside the chamber. Samples of air inside the chamber were collected monthly in periods of 0, 1, 2 and 4 minutes to determine the gas flow, during the day (between 10:00 – 12:00 hours) and at night (between 22:00 – 24:00 hours). To capture the bubbles (emission), glass fiber funnels suspended by floats were installed on the surface of ponds. We connected a graduated bottle at the extremity of the funnels to trap the bubbles released within 24 hours. The air samples from both methods were placed in transfer tubes for analysis by gas chromatography with TCD detector (Thermal Conductivity Detector from Construmaq, São Carlos, Brazil). The final value corresponds to the mass of carbon absorbed (input) or emitted (output) during daytime and nighttime throughout the experiment.

Retained C in periphyton was analyzed by samples of 10 cm width by 20 cm height collected from the additional substrates inside the net fences (between 20 and 40 cm from the water surface). We extracted the periphyton from the substrates using an ultrasonic homogenizer (USC – 750, Unique) according to Thompson et al. (2002). The samples were dried (AOAC, 1995 - 934.01) and the total C content was analyzed by combustion in high temperature and conversion of samples into gases (CHNS Elementar - Vario Macro Cube with Thermal Conductivity Detector sensor). From the dry mass sample, we estimated the total mass adhered on the substrates. Then, to calculate the retained C in the substrate, we multiplied the total mass by C content in the sample.

Sediment samples were collected by tripton samplers (six 1.876 L PVC tubes, with 9.7 cm diameter and 25.4 cm long to 15 cm depth). One tripton sampler was placed on the bottom of each pond for 48 h biweekly. To determine the C content, the samples were dried (AOAC, 1995 - 934.01) and analyzed by muffle furnace method (AOAC, 1995 - 920.153). From the total amount of sediment sampled in 48h considering the area of the tripton's sampler (~0.045 m²), we estimated the total amount of sediment accumulated in the pond. Then, to calculate the total C in sediment, we multiplied the accumulated mass by C content in the sample. Organic matter was determined by incinerating samples in a muffle furnace at 550º C (AOAC 1995 - 920.153).

Data analyses

Survival data was square root arcsine transformed prior to analysis. All data were tested for normality (Shapiro–Wilk) and homoscedasticity (Levene). As both conditions were satisfied, analysis of variance (One-Way ANOVA) was applied to verify the differences in variables of water quality, productions, and compartments of phosphorus budget. Productivity data of prawns were not normal and, therefore, were analyzed with Kruskal-Wallis test. Statistical analyses were carried out in R software (version 0.98.945) and the level of significance considered was $\alpha = 0.05$. When significant differences were detected among treatments, means were compared by Tukey's test.

Results

Carbon loads and the comparisons among treatments are shown in Table 3. Carbon percentages are represented as flow diagrams in Figures 1, 2, and 3. The total input values were higher than the output in all treatments. Thus, the unaccounted portion of carbon was identified in the output. Diet was the major input of carbon in all treatments, ranging from ~54 to 63%. The second most representative input was the inlet water, ranging from ~29 to 39%, and then, the absorbed gases with a contribution ranging from ~5 to 7%. The other input compartments together ranged from 1.3 to 1.4%. No statistical differences were found among the treatments in the input compartments.

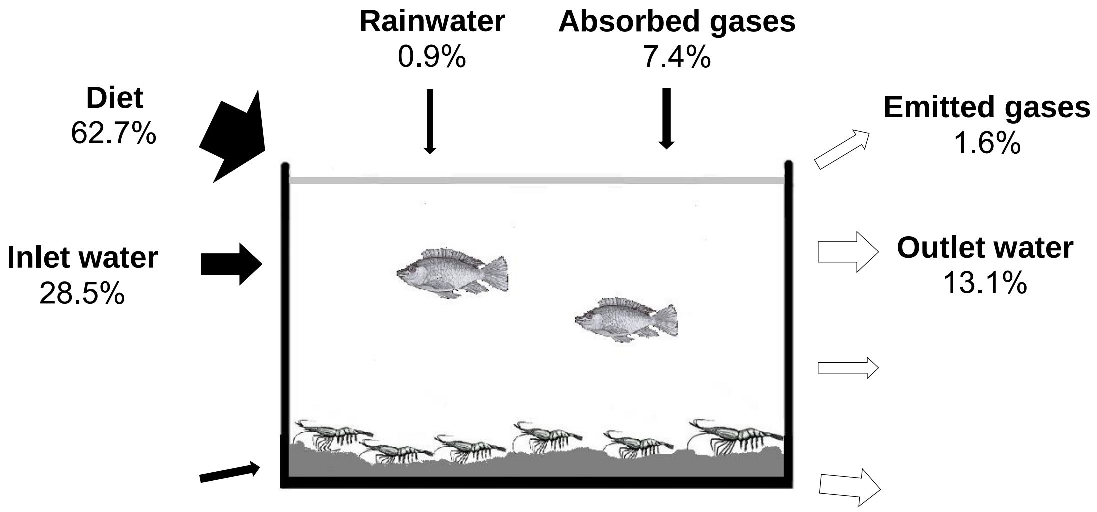
The budget showed that most of carbon that entered the system was deposited in the sediment, ranging from ~38 to 70%. The organic matter (OM) accumulated in the sediment accounted for ~34 to 43% of the dry matter and was significantly lower in periphyton-based treatments. Carbon retained (recovered) in reared animals and periphyton ranged from ~13 to 14% (being 10.9 to 12.0% in fish; 1.0 to 1.2% in prawns; 1.2% in periphyton). Carbon outputs by the outlet water ranged from ~12 to 13%, by emitted gases from ~2 to 3% and by dead fish from 1 to 2%. Only harvested fish significantly differed among treatments, being significantly higher in GS and lower in BS; both treatments did not differ from control group (WS). The final budget showed that the unaccounted carbon ranged from ~1 to 32% and BS treatment had the major portion unidentified.

Table 3. Means (±SD) of carbon budget in IMTA* system with *O. niloticus* and *M. amazonicum* obtained from the following treatments: without substrate (WS), geotextile substrate (GS) and bamboo substrate (BS). Different letters in the same line indicate significant differences between treatments (*P*<0.05).

Compartments	**Treatments**		
(kg/ha)	**WS**	**GS**	**BS**
Input			
Diet	3,874.7 ± 399.7	3,746.6 ± 221.2	3,637.3 ± 333.6
Inlet water	1,758.4 ± 263.4	2,090.6 ± 678.4	2,643.0 ± 916.0
Rainwater	55.1 ± 0.0	55.1 ± 0.0	55.1 ± 0.0
Absorbed gases	455.3 ± 157.7	323.4 ± 98.3	411.7 ± 94.8
Stocked fish	31.5 ± 2.2	31.3 ± 4.9	30.4 ± 1.6
Stocked prawns	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.0
Output			

Outlet water	808.9 ± 381.9	761.3 ± 192.1	804.2 ± 281.1
Emitted gases	98.1 ± 94.1	209.8 ± 96.6	223.5 ± 44.7
Periphyton	-	77.2 ± 9.6	80.8 ± 23.5
Harvested fish	727.1 ± 42.2^{ab}	751.0 ± 120.6^{a}	735.5 ± 83.2^{b}
Harvested prawns	63.9 ± 16.4	68.7 ± 20.8	80.9 ± 3.9
Dead fish	123.6 ± 44.7	77.0 ± 7.4	66.0 ± 18.2
Sediment	4,308.6 ± 1,209.8	3,451.3 ± 932.7	2,606.4 ± 726.6
Unaccounted			
Input - Output	143.7 ± 739.3^{b}	1,097.3 ± 560.1^{b}	2,405.5 ± 321.9^{a}

*IMTA: Integrated Multi-trophic Aquaculture.



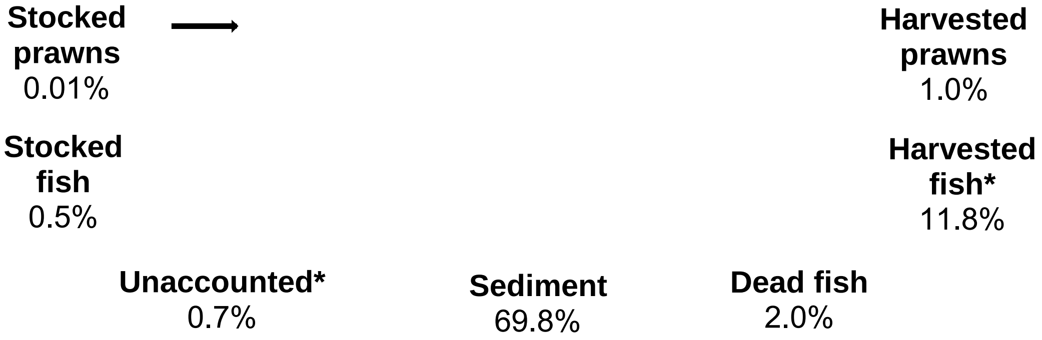
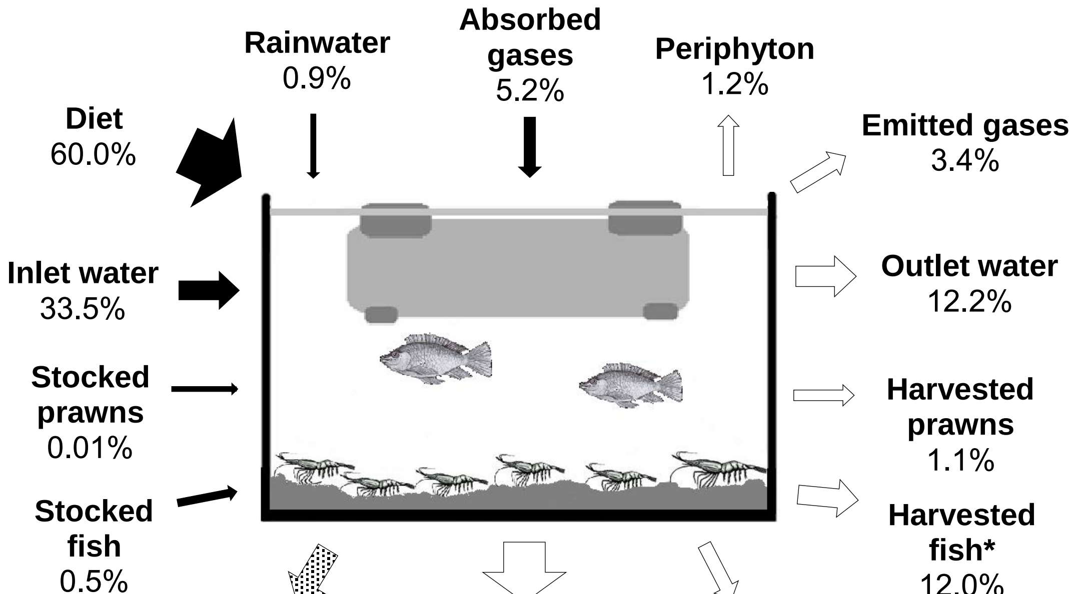


Figure 1. Carbon budget in integrated multi-trophic aquaculture system with *O. niloticus* and *M. amazonicum* in treatment without substrate (WS). Values are shown in percentages based on the total input to pond. *Indicate significant differences (P<0.05) among treatments.



Dead fish
1.2%

Figure 2. Carbon budget in integrated multi-trophic aquaculture system with *O. niloticus* and *M. amazonicum* in treatment with substrate made of geotextile blanket (GS). Values are shown in percentages based on the total input to pond. *Indicate significant differences ($P<0.05$) among treatments.

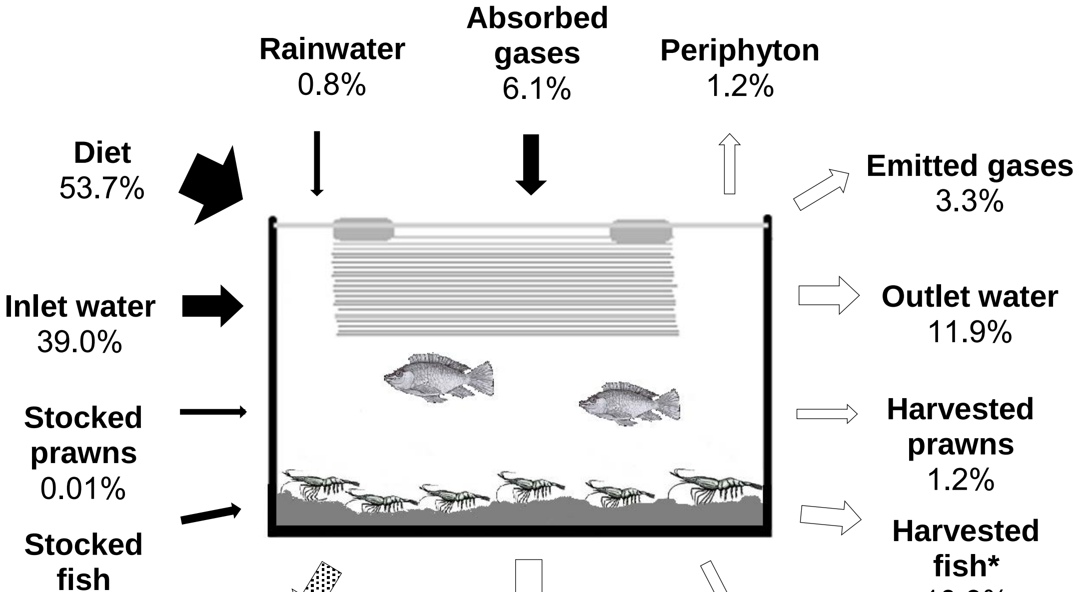


Figure 3. Carbon budget in integrated multi-trophic aquaculture system with *O. niloticus* and *M. amazonicum* in treatment with substrate made of bamboo (BS). Values are shown in percentages based on the total input to pond. *Indicate significant differences (P<0.05) among treatments.

Discussion

Diet was the major input of carbon in this prawn/fish IMTA system (~54 to 63%). This bulk of organic carbon (OC) through the diet was expected, since previous studies on nutrient budgets have demonstrated that feed is the single largest source of OC in prawn monocultures, ranging from ~90 to 94% (Sahu et al., 2013b; Adhikari et al., 2014), and in integrated system of fish farming, ranging from 85 to 92% (Gal et al., 2013). The proportion found in this study, however, is lower because of the substantial contribution of the inlet water.

Water supply had the second highest contribution on the total inputs (~29 to 39%). This contribution is higher than reported in literature for cultures with no water exchange, which ranges from 0.8 to 1.2% (Sahu et al., 2013a; 2013b; Adhikari et al., 2014). The inlet water used to fill the ponds is nutrient-rich (hypereutrophic water from a reservoir that receives aquaculture effluents), which contributed to the high amount of carbon that entered in the system. This is a feasible alternative to reuse anthropogenic nutrient loads and, when available, may represent a source of unpaid nutrients that can decrease the chemical fertilizers dependency of aquaculture systems (Fernando and Halwart, 2000) and be incorporated into the biomass of reared (Kimpara et al., 2011). In addition, the volume used to replace loss by seepage and evaporation (~9.5% daily) enhanced this proportion.

A small fraction of carbon inputs was recovered in harvested animals (~13 to 14% OC), while the largest fraction was lost to others compartments. The addition of substrates did not affect significantly the performance of prawns to retain OC. Nonetheless, this approach affect significantly the OC retention in fish. Our results show that Nile tilapia retains more OC when reared in ponds with substrates made of geotextile blanket (GS) than with substrates made of bamboo (BS), but both did not differ from tilapias reared in control group (WS). This difference, however, did not affect system's efficiency as we expected, since the increase of OC in fish tissues was not followed by the increase in fish biomass (Table 2). The retained portion of OC in fish biomass (~11 to 12%) can be regarded as an average of what is found in the literature, which is reported to be about 6% in fish polyculture (Nhan et al., 2008), 9% in Catfish monoculture (Boyd, 1985) and 16% in Carp monoculture (Avnimelech and Lacher, 1979). The retained portion of OC incorporated into prawn biomass (mean of 1.1%), however, was lower than in monoculture systems, that is reported to be from ~10 to 15% (Sahu et al., 2013a; 2013b; Adhikari et al., 2014). Nonetheless, prawns reared in monoculture systems are fed with specific diets while in the present study are co-cultured animals, which feeds on wastes and periphyton. This difference in feed leads to distinct OC retention; however, retained portions in co-cultured species are extra gains of nutrients. The stocking density used for prawns (21.5 individuals/m^2) may also have contributed for low OC incorporation. It is known that nutrient recovery increases as the system is intensified (Sahu et al., 2013a) and that *M. amazonicum* tolerates the increased stocking density in the grow-out phase at least until 80 PL/m^2 (Moraes-Valenti and Valenti, 2007). This indicates that increased densities of prawns should be tested in this IMTA model, optimizing the nutrient loading of the system.

Entrapped carbon in periphyton was low (1.2%) and did not differ between treatments. Fluctuations of periphyton population occur throughout the culture because of the processes of ecological succession and detachment on substrates (Saikia, 2011). In addition, in cultures of voracious grazers, such as tilapia, the rate of feeding is more intense than the time taken for periphyton recolonize (Ranjeet and Hameed, 2015). As analized samples of periphyton were collected at harvest, this data may not represent the entire assimilation of carbon during the culture. Nonetheless, the difference of OC found in fish tissues suggests that periphyton performs better in substrates made of geotextile blanket than in substrates made of bamboo. The amount of periphyton dry mass (DM) attached on substrates at harvest (12.7 ± 5.7 g DM/m² for BS and 40.7 ± 13.8 g DM/m² for GS) indicates that, probably, substrates made of geotextile blanket may have provided available periphyton for prolonged duration in spite of continuous grazing by tilapias. This difference could be attributed to the higher porosity of geotextile blanket, increasing periphyton conditions to be attached. This trend was also observed by Ranjeet and Hameed (2015) in substrates made of non-degradable fibers wrapped on PVC pipes, which are more porous and thick compared to bamboo surfaces. Nonetheless, animal grazing and satiation after feeding should be analyzed in future studies to better understand if the carbon trapped within the pores of geotextile blanket substrate can really be assimilated by the reared animals.

The release of carbon through outlet water, represented by the organic and inorganic compounds, ranged from ~12 to 13% and did not differ among treatments. On average, 77% of released carbon in outlet water was organic. This variation is above the range reported in prawn monocultures ~2 to 3% OC (Sahu et al., 2013a; 2013b; Adhikari et al., 2014). Nonetheless, the inlet water used in those studies had fewer nutrients, which contributed to the outlet water be less representative. The addition of substrates did not entrap nutrients enough to decrease the amount of carbon in the outlet water as we expected. Thus, as the outlet water remains nutrient-rich, the most rational alternative to preserve the health of adjacent water bodies is conduct this effluent to cultures into rice fields and gardens (Phan et al., 2009).

Most of the OC not recovered in harvested animals and periphyton was found in the pond bottom (~55 to 70%), with no significant differences among treatments. This result is normally observed in extensive, semi-intensive and intensive ponds (Avnimelech and Ritvo, 2003) and indicates that studies to increase the reuse of accumulated nutrients in the sediment are the path to the success of aquaculture production systems. The OC accumulation in the sediment is around 64% (Sahu et al., 2013a; 2013b; Adhikari et al., 2014) in prawn monoculture and 81% (Nhan et al., 2008) in fish polyculture. These high concentrations are because organic matter from the water column settles to the sediment surface and may accumulate at a rate greater than losses from decomposition (Steeby et al., 2004). In the present study, organic matter sedimentation was significantly lower in periphyton-based treatments, showing that the presence of periphyton changes water-sediment relationships in ponds. Periphyton may interfere in the dynamics of cultivation by trapping nutrients from the water column (Milstein et al., 2003) and, consequently, reducing particle sedimentation (van Dam et al., 2002). High accumulation of organic matter in pond sediments causes severe oxygen depletion at the water-sediment interface (Boyd, 1990). Under anoxic conditions, organic compounds are often decomposed to H_2S, NH_3 and CH_4, which are harmful or even lethal to reared animals (Kassila, 2003), mainly to prawns that are directly exposed to the sediment conditions. Therefore, periphyton contributes to maintain the benthic ecosystem under aerobic metabolic pathways.

Gas exchange of this IMTA system with the atmosphere showed higher carbon inputs (~5 to 7%) than outputs (~2 to 3%), with no significant differences among treatments. This indicates that, in general, the adopted IMTA system absorbs carbon from atmosphere and that the addition of substrates does not influence the emission/absorption of this element. This result is interesting especially because aquaculture has been criticized for the impacts caused to the environment. In recent years, one of the main concerns regarding aquaculture systems are the global greenhouse gases (GHGs) emissions (Williams and Crutzen, 2010; Hu et al., 2012). The main GHGs related to this element are carbon dioxide (CO_2) and methane (CH_4), both emitted through organic materials decomposition process (Hu et al., 2012). Perhaps, the better use of accumulated nutrients observed in this prawn/fish IMTA system contributed to the low emission of GHGs. In addition, the use of nutrient-rich water may have contributed to carbon absorption through primary productivity, which in aquaculture ponds is reported around 2 g C/m²/d (Boyd, 1973; Yusoff and McNabb, 1989; Knud-Hansen et al., 1991). The result found in this prawn/fish IMTA system is an environmental benefit, which should be further investigated as a carbon credit, stimulating systems that recover carbon from the atmosphere.

Unidentified portion of carbon ranged from ~1 to 32% and was significantly higher in BS treatment. Earlier studies indicate percentages ranging from 17 to 19% (Sahu et al., 2013a; 2013b; Adhikari et al., 2014). Unaccounted portions are common in mass balances and, in this prawn/fish IMTA system, the unaccounted carbon in the output may be related mainly to loss by seepage, incorporation in small fish (not computed), migration of animals that can enter in the system, deposit nutrients, and then leave the system, and even caused by methodological errors.

In conclusion, this study provides quantitative information on carbon budget that are valuable to reconsider aquaculture practices. We have characterized the main attributes of the carbon budget of prawn/fish IMTA systems with and without addition of substrates, which can support future efforts toward improve conversion of inputs in harvestable products. Our results showed that the addition of substrates in the system promoted different organic carbon retentions in fish tissues, but with no improvement on system efficiency. Further attention on periphyton development and its grazing by reared animals has to be considered in future researches in order to understand the lack of treatment effects on system efficiency. In addition, atmospheric carbon sequestration showed a positive externality of aquaculture, which should be investigated in carbon credits studies. Some adjustments should be tested in this system to improve its results, such as increase the stocking density of prawns, reduce the feed rate to force the animals to eat periphyton and wastes, add other detritivore-iliophagus (mud eating) species in the system and use sediment as fertilizer.

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CAPÍTULO 5

CONCLUSÕES GERAIS

- A adição de substratos em sistemas multitróficos e multiespaciais de tilápia-do-nilo e camarão-da-amazônia teve pouco efeito na reciclagem de nitrogênio (N), fósforo (P) e carbono (C). Em geral, o uso de substratos gerou benefícios como a redução de matéria orgânica no sedimento e redução de fósforo na água de despesca. No entanto, não melhorou a conversão de N, P e C em biomassa animal.
- A falta de efeito dos substratos sobre a conversão de nutrientes em biomassa animal indica baixa produtividade e variação sucessional do perifiton. Provavelmente, algum fator ambiental prejudicou o desenvolvimento da comunidade perifítica ou então a área de substrato utilizada não foi suficiente para a colonização do montante necessário à alimentação dos animais cultivados.

- Em geral, a maioria dos nutrientes que entraram no sistema foram perdidos para o ambiente. As principais entradas ocorreram através da dieta (~66% N, ~56% P e ~59% C orgânico) e da água de abastecimento (~31% N, ~24% P e ~34% C total). A perda de nutrientes para o ambiente deu-se principalmente pelo acúmulo no sedimento (~31% N, ~64% P e ~54% CO) e, no caso do nitrogênio, também pela liberação na forma de N_2 (~28-36%).
- A recuperação de nutrientes pelas tilápias (~20% N, ~21% P e ~12% CO) pode ser considerada satisfatória em comparação com outros balanços de nutrientes disponíveis na literatura.
- A recuperação de nutrientes pelos camarões (2,4% N, 1,6% P e 1,1% CO) pode ser considerada como um ganho de nutrientes para o sistema, pois é resultado do reaproveitamento de nutrientes.
- O mapeamento de N, P e C mostrou o destino dos recursos investidos em sistemas multitróficos e multiespaciais de tilápia-do-nilo e camarão-da-amazônia e gerou informações que podem contribuir para o entendimento da ciclagem desses nutrientes em viveiros. Além disso, apontou fatores que podem aumentar a eficiência destes sistemas em pesquisas futuras, tais como: análise da produtividade de perifiton, análise do consumo de perifíton pelos animais cultivados, aumento da densidade de estocagem dos camarões, adição de outras espécies que ingerem detritos e redução da oferta de ração para forçar os animais a comer perifíton e resíduos.