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CENTRO DE AQUICULTURA DA UNESP

EFICIÊNCIA NO USO DE NUTRIENTES EM CULTIVOS INTEGRADOS DE PEIXES E CAMARÕES

Dalton Belmudes Neto

Jaboticabal, São Paulo
2024

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Jaboticabal, 27 de fevereiro de 2024

SUMÁRIO

DEDICATÓRIA

“A mente que se abre a uma nova ideia jamais volta ao seu tamanho original.”

(Albert Einstein)

Dedico a minha família e a minha esposa que me acompanharam ao longo dessa jornada e me motivaram a alcançar os meus objetivos.

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Gostaria de agradecer meus PAIS por todo amparo necessário para a minha formação, com esforços intermináveis, ensinamentos que a universidade não nos oferece e por toda educação e respeito que me ensinaram a ter com qualquer pessoa e todo lugar.

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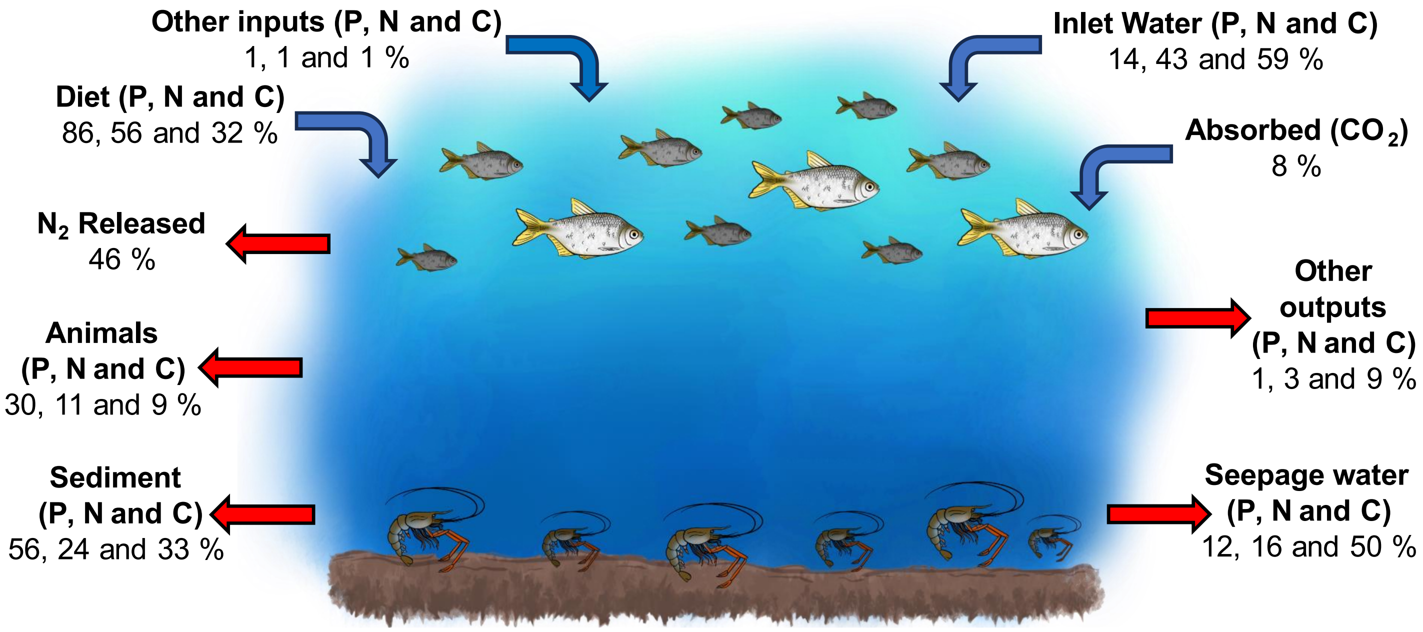
GRAPHICAL ABSTRACT

LP treatment – Inputs and Outputs of Phosphorus (P), Nitrogen (N) and Carbon (C)



Other inputs (P, N and C)
1, 1 and 1 %

Diet (P, N and C)
86, 56 and 32 %

Inlet Water (P, N and C)
14, 43 and 59 %

Absorbed (CO_2)
8 %

N_2 Released
46 %

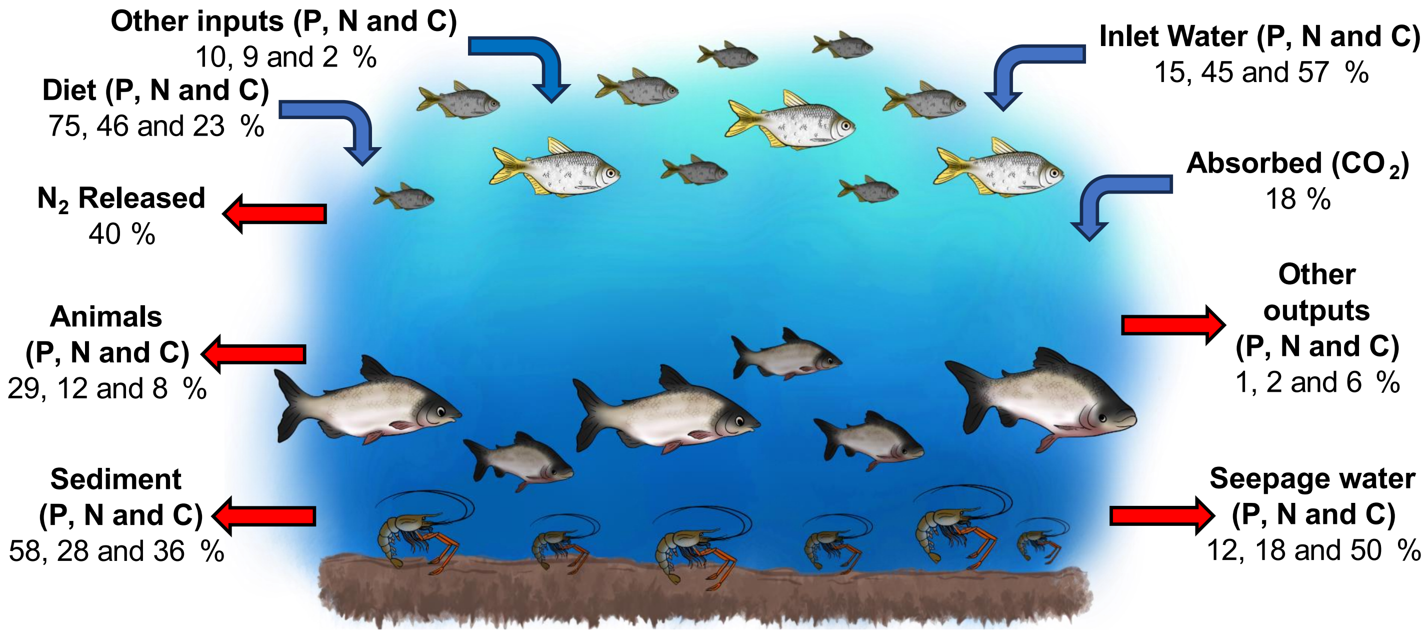
Animals (P, N and C)
30, 11 and 9 %

Other outputs (P, N and C)
1, 3 and 9 %

Sediment (P, N and C)
56, 24 and 33 %

Seepage water (P, N and C)
12, 16 and 50 %

LPC treatment – Inputs and Outputs of Phosphorus (P), Nitrogen (N) and Carbon (C)



Other inputs (P, N and C)
10, 9 and 2 %

Diet (P, N and C)
75, 46 and 23 %

Inlet Water (P, N and C)
15, 45 and 57 %

Absorbed (CO_2)
18 %

N_2 Released
40 %

Animals (P, N and C)
29, 12 and 8 %

Other outputs (P, N and C)
1, 2 and 6 %

Sediment (P, N and C)
58, 28 and 36 %

Seepage water (P, N and C)
12, 18 and 50 %

RESUMO

Nos últimos anos o cultivo integrado tem sido utilizado na aquicultura para aumentar a sustentabilidade, podendo otimizar o uso de recursos, espaço e água. Esse trabalho teve como objetivo avaliar a eficiência no uso de nutrientes em viveiros de terra, com adição de espécies de habitat bentônico. Para isso, foi desenvolvido um experimento em doze viveiros, com dois tratamentos e 6 repetições: 1) lambari-do-rabo-amarelo (*Astyanax lacustris*) e camarão-da-malásia (*Macrobrachium rosenbergii*) (LP) 2) lambari-do-rabo-amarelo, camarão-da-malásia e curimbatá (*Prochilodus lineatus*) (LPC). O lambari (espécie-alvo) foi alimentado com dieta equivalente a 10% de sua biomassa. O curimbatá e o camarão se beneficiaram dos nutrientes e de resíduos desta dieta. Foram coletadas amostras do sedimento, da água, dos gases trocados com a atmosfera, da ração ofertada ao lambari e dos animais povoados e despescados para a quantificação dos nutrientes. Respectivamente, nos tratamentos LP e LPC, o fósforo entrou no sistema principalmente por meio da dieta ~86,1 e 75%. A maior parte do fósforo que entrou no sistema se acumulou no fundo dos viveiros como sedimento ~55,9 e 57,6% e na biomassa dos peixes ~30,3 e 29,4%. O nitrogênio entrou no sistema principalmente por meio da dieta ~56,4 e 46,4% e da entrada de água ~43,4 e 45%. A saída de nitrogênio do sistema ocorreu principalmente como N_2 liberado por bolhas ~46,3 e 40,3% e acumulado no sedimento ~23,9 e 27,9%. O carbono entrou no sistema principalmente por meio da entrada de água ~59,3 e 57,3%, seguido pela dieta ~32,1 e 22,9%. A saída de carbono ocorreu principalmente pela água de infiltração ~49,5 e 49,7%, seguida do acúmulo no sedimento ~33,1 e 35,6%. Os dados sugerem que os nutrientes entram principalmente por meio da dieta (fósforo e nitrogênio) e da água de abastecimento (carbono), e os cultivos integrados retêm uma parcela desses nutrientes no sistema. No caso do fósforo boa parte é acumulado na biomassa dos animais despescados. Portanto, a aquicultura integrada apresenta potencial para otimizar o uso de nutrientes.

Palavras-chave: balanço de nutriente, cultivo integrado, lambari-do-rabo-amarelo, camarão-da-malásia, curimbatá.

ABSTRACT

In recent years, integrated culture has been used in aquaculture to increase sustainability, optimizing the use of resources, space, and water. This work aimed to evaluate the efficiency in the use of nutrients in soil ponds, with the addition of benthic habitat species. For this, an experiment was developed in twelve ponds, with two treatments and 6 replications: 1) yellow tail lambari (*Astyanax lacustris*) and giant river prawn (*Macrobrachium rosenbergii*) (LP) 2) yellow tail lambari, giant river prawn and curimbatá (*Prochilodus lineatus*) (LPC). The lambari (target species) was fed a diet equivalent to 10% of its biomass. Curimbatá and prawn benefited from the nutrients and residues of this diet. Samples were collected from sediment, water, gases exchanged with the atmosphere, from the feed offered to lambari and from stocked and harvested animals for the quantification of nutrients. Respectively, in the LP and LPC treatments, phosphorus input the system mainly through the diet ~86.1 and 75%. Most of the phosphorus that input the system accumulated at the bottom of the pond as sediment ~55.9 and 57.6% and in fish biomass ~30.3 and 29.4%. Nitrogen input the system primarily through diet ~56.4 and 46.4% and water inlet ~43.4 and 45%. Nitrogen output from the system occurred primarily as N2 released by bubbles ~46.3 and 40.3% and accumulated in the sediment ~23.9 and 27.9%. Carbon input the system primarily through water inlet ~59.3 and 57.3%, followed by diet ~32.1 and 22.9%. Carbon output occurred mainly through seepage water ~49.5 and 49.7%, followed by accumulation in the sediment ~33.1 and 35.6%. The data suggests that nutrients input mainly through the diet (phosphorus and nitrogen) and water inlet (carbon), and integrated cultures retain a portion of these nutrients in the system. In the case of phosphorus, much of it is accumulated in the biomass of harvested animals. Therefore, integrated aquaculture has the potential to optimize nutrient use.

Keywords: nutrient budget, integrated culture, yellow tail lambari, giant river prawn, curimbatá.

CAPÍTULO 1

INTRODUÇÃO, APRESENTAÇÃO E JUSTIFICATIVA DO TRABALHO

Em 2020, a pesca e a produção aquícola atingiram um recorde histórico de 214 milhões de toneladas, valor de cerca 424 mil milhões de dólares. A produção de animais aquáticos em 2020 foi 60% maior do que a média da década de 1990 (FAO, 2022). A crescente demanda global e a industrialização da atividade pode aumentar a quantidade de recursos não utilizados, as emissões de gases de efeito estufa e a eutrofização dos ambientes aquáticos, principalmente a partir da expansão e intensificação dos sistemas de aquicultura que usam alimento alóctone (Clark e Tilman, 2017; Flickinger *et al*., 2020a). Assim, a realização da atividade com embasamento científico para aumentar a eficiência dos sistemas pode aumentar a sustentabilidade, desencadeando um maior aproveitamento dos recursos (Boyd e Zimmerman, 2010; Valenti *et al*., 2018).

Uma estratégia utilizada nos últimos anos na tentativa de um desenvolvimento sustentável é o cultivo integrado. Este pode ser definido como a criação de duas ou mais espécies que compartilhem os recursos disponíveis de forma complementar. Essa forma de cultivo possibilita a alimentação apenas da espécie alvo enquanto uma ou mais espécies secundárias utilizam os nutrientes, resíduos e subprodutos originados pela espécie alvo (Chopin *et al*., 2013; Marques *et al*., 2016). Essa estratégia otimiza o uso do espaço, da água e de outros recursos naturais, tais como energia e fontes de nitrogênio, fósforo e carbono (Diana *et al*., 2013; Marques *et al.*, 2016). Assim, os sistemas de aquicultura integrados são uma estratégia para melhorar a sustentabilidade ambiental.

O cultivo em sistema integrado de lambari-do-rabo-amarelo (*Astyanax lacustris*), camarão-da-malásia (*Macrobrachium rosenbergii*) e curimbatá (*Prochilodus lineatus*) mostra-se promissor nesse contexto. O lambari-do-rabo-amarelo nada ativamente na coluna d’água, têm hábitos alimentares onívoros oportunistas e têm um grande potencial para produção de forma sustentável. Esta espécie é encontrada no alto Paraná, Bacias Hidrográficas do Paraguai, Tocantins, São Francisco e Rio Doce (Lima *et al*., 2003; Lucena e Soares, 2016; Fonseca *et al*., 2017). A reprodução ocorre durante todo o ano, mas em taxas mais elevadas na primavera e no verão, sugerindo que o aumento da temperatura e da precipitação aumenta o desempenho reprodutivo (Rodrigues *et al*., 1992; Sato *et al*., 2006; Fonseca *et al*., 2017). O cultivo dessa espécie tem crescido muito no Estado de São Paulo nos últimos anos, impulsionado pela indústria de iscas vivas para a pesca esportiva. A lambaricultura apresenta uma produção estimada que ultrapassa 1.000 toneladas por ano (Valenti *et al*., 2021). Esses peixes são produzidos principalmente em monocultivos realizados em viveiros de fundo natural, usando níveis de tecnologia variados (Gonçalves *et al*., 2014; Fonseca *et al*., 2017). No entanto, os lambaris apresentaram grande potencial para serem cultivados em conjunto com outras espécies (Marques *et al*., 2021)

O camarão-da-malásia é epibentônico e se alimenta principalmente de detritos e organismos bentônicos (New e Valenti, 2000), apresentando potencial de inserção em cultivos integrados para otimizar o uso de materiais e energia gerados pelos peixes de coluna d'água. A produção de camarão-da-malásia (*Macrobrachium rosenbergii*) representa cerca de 50% em valor e 45% em volume da produção global de camarões (David, et al., 2018). A cultura desta espécie é uma importante atividade econômica em vários países em todo o mundo, principalmente na Ásia. Na América, esta atividade tem importância local em alguns países, como Estados Unidos e Brasil (Ming, 2014; Banu e Cristão, 2016; David, *et al*., 2018). Estudos anteriores mostraram que o cultivo integrado de camarão e lambari foi compatível e apresentou aumento na rentabilidade (Rodrigues, 2017). Utilizar instalações e recursos naturais de forma mais eficiente, é possível reduzir o custo de produção e aumentar sustentabilidade econômica e ambiental (Valenti, *et al*., 2018). Assim, a introdução do camarão-da-malásia apresenta possibilidade de aliar potencial de mercado e aproveitamento de recursos.

Por outro lado, estudos anteriores mostraram a ocorrência de quantidade elevada de nutrientes acumulada no fundo dos viveiros ao final dos cultivos integrados de peixes e camarões (Flickinger *et al*., 2019, 2020a, 2020b; David *et al*., 2017a, 2017b, 2021). Isso sugere que esses sistemas têm capacidade para alimentar mais uma espécie iliófaga nos mesmos viveiros. A inclusão do curimbatá no cultivo integrado de tambaquis (*Colossoma macropomum*) e camarões-da-amazônia (*Macrobrachium amazonicum*) mostrou resultados bastante promissores (Franchini *et al*., 2020). Esta espécie se alimenta de detritos orgânicos, fauna bentônica e ração e possui importância ecológica e ambiental em teias alimentares aquáticas. Isso permite a sua integração em sistemas de policultura (Castagnolli, 1992; Murgas *et al*., 2012; Zuffo *et al*., 2021). O curimbatá é comercializado principalmente para consumo humano e para iniciativas de repovoamento de bacias no sudeste do Brasil (Valenti *et al*. 2021). A diversificação dos produtos e dos mercados, bem como a eficiência no uso de nutrientes e redução da poluição aumentam a sustentabilidade dos sistemas de produção aquícola (Valenti *et al*., 2018).

No entanto, a melhoria da eficiência de sistemas integrados exige um conhecimento detalhado da ciclagem de nutrientes. É essencial entender como os nutrientes são distribuídos nos vários compartimentos ecológicos dentro dos viveiros para melhoria do sistema e para direcionar seu acúmulo nas espécies cultivadas (David *et al*., 2017b). O primeiro passo para entender a ciclagem de nutrientes em sistemas de aquicultura é quantificar alguns elementos-chave como o fósforo, o nitrogênio e o carbono em cada compartimento (Mariscal-Lagarda e Páez-Osuna 2014; David *et al*., 2017a). O fósforo é um nutriente fundamental e o principal nutriente limitante nos ecossistemas de água doce (Boyd, 2020). No entanto, o rápido aumento da demanda humana por alimentos quadruplicou seus insumos, evidenciando a necessidade do uso eficiente. A escassez futura de fósforo pode ameaçar a produção global de alimentos (Huang *et al*., 2020). Assim, o gerenciamento do ciclo desse nutriente na água tem potencial de aumentar a sustentabilidade da aquicultura (Flickinger *et al*., 2020b), podendo auxiliar na melhoria da eficiência cultural com redução do desperdício e otimização da gestão de alimentos (Marques *et al*., 2016; David *et al*., 2017a).

O nitrogênio é um elemento-chave porque faz parte das proteínas constitutivas e enzimas de todos os organismos. Esse nutriente é essencial na dieta de qualquer animal, e seu metabolismo gera produtos de excreção, tais como as diferentes formas de nitrogênio amoniacal, que estão entre os principais poluentes dos ambientes aquáticos (Valenti *et al*., 2018). Assim, compreender o balanço de nitrogênio é um pré-requisito para alcançar a redução de resíduos (Mariscal-Lagarda e Paez-Osuna, 2014), diminuir o uso fertilizantes químicos e principalmente otimizar o uso das dietas comerciais em fazendas de aquicultura.

O carbono é um nutriente fundamental na aquicultura, estando presente na dieta de muitos animais e podendo ser convertido em biomassa colhida. As parcelas não incorporadas pelos animais podem acumular no fundo dos viveiros por meio de ração ofertada não aproveitada, fezes e outros processos de sedimentação (Boyd, 2020). O restante é convertido em gás carbônico (CO_2), que melhora a produção primária e permite a expansão da cadeia alimentar aquática, o que leva a um aumento na rotatividade do fitoplâncton (Moriarty, 1997; Boyd *et al*., 2010). O fitoplâncton morto e outros materiais orgânicos retornam aos sedimentos do fundo, originando a decomposição anaeróbica, que pode causar a liberação de metabólitos prejudiciais (Boyd *et al*., 2020; Flickinger *et al*., 2020a). Portanto, o conhecimento do balanço de carbono é necessário para a compreensão do acumulo desse nutriente nos sistemas de aquicultura e para entender como as espécies cultivadas podem converter esse nutriente essencial em biomassa colhida (Liu *et al*., 2014; Marques *et al*., 2016; Flickinger *et al*., 2020a).

Desse modo, o presente trabalho visou obter informações sobre o balanço de fósforo, nitrogênio e carbono em sistemas de produção aquícola. Foi descrito o balanço de nutrientes no cultivo integrado de lambari-do-rabo-amarelo com o camarão-da-malásia e com o camarão-da-malásia mais o curimbatá. Dado que a maioria dos nutrientes se acumula no fundo dos viveiros e que em estudos anteriores a adição de uma espécie de camarão não se mostrou suficiente para aproveitar todo esse material (Flickinger *et al*., 2019, 2020a, 2020b; David *et al*., 2017a, 2017b, 2021), nesse trabalho foi adicionado também o curimbatá para avaliar se ocorre aumento na eficiência da assimilação desses nutrientes utilizando espécies de interesse econômico. Esse projeto está inserido na Rede de Pesquisa Sustentabilidade na Aquicultura, que vem sendo apoiada por várias agências de fomento a pesquisa (Edital MCT / CNPq / MEC / CAPES / CT AGRO / CT HIDRO / FAPS / EMBRAPA Nº 22/2010 – REPENSA; CNPq Processo 562820/2010-8; FAPESP Processo 10/52210-3; CNPq Processos; 406069/2012-3 e 306361/2014-0; CAPES-EMBRAPA edital 15/2014 projeto número 24; FINEP convênio número 01.10.0578.00/10), a qual tem como objetivo a análise da sustentabilidade ambiental, econômica e social dos principais sistemas de produção aquícolas usados no Brasil. Portanto, nesse projeto foram realizadas análises modernas, importantes para a sustentabilidade ambiental e para subsidiar pesquisas futuras.

OBJETIVO GERAL

Testar a hipótese de que a eficiência no aproveitamento de fósforo, nitrogênio e carbono em viveiros de terra é potencializada pela introdução de espécies de habitat bentônico. Este estudo adotou o lambari-do-rabo-amarelo, o camarão-da-malásia e o curimbatá como modelos.

OBJETIVOS ESPECÍFICOS

- Quantificar o acúmulo de fósforo, nitrogênio, carbono nos vários compartimentos dos sistemas do cultivo integrado de lambari-do-rabo-amarelo (*Astyanax lacustris*) com o camarão-da-malásia (*Macrobrachium rosenbergii*), e com o camarão-da-malásia mais o curimbatá (*Prochilodus lineatus*);
- Avaliar se a inclusão dos curimbatás no cultivo de lambaris interfere na assimilação de fósforo, nitrogênio e carbono pelos lambaris.

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CAPÍTULO 2

PHOSPHORUS BUDGET OF *Astyanax lacustris* FARMING IN INTEGRATED CULTURE WITH *Macrobrachium rosenbergii* AND WITH *Macrobrachium rosenbergii* AND *Prochilodus lineatus*

ABSTRACT

The present work aimed to map the phosphorus content in the main ecological compartments of earthen ponds of integrated cultures of yellow tail lambari (*Astyanax lacustris*) and giant river prawn (*Macrobrachium rosenbergii*) (LP) in comparison with yellow tail lambari, giant river prawn and curimbatá (*Prochilodus lineatus*) (LPC). The experimental design was completely randomized, with two treatments and six replications. Phosphorus inputs the system mainly through the diet (86.1% in LP and 75% in LPC), inlet water (13.6% in LP and 15.3% in LPC) and in stocked curimbatás (9.6% in LPC). Most of the phosphorus that entered the system accumulated at the bottom of the pond as sediment (55.9% in LP and 57.6% in LPC) and in fish biomass (30.3% in LP and 29.4% in LPC). The rest of the phosphorus infiltrated the soil and came out in the harvesting water. The data suggests that much of the phosphorus that inputs the system was accumulated in the biomass of captured animals. Furthermore, both treatments have a great capacity for retaining and recovering phosphorus from nutrient-rich aquatic environments. Therefore, the addition of benthic species with complementary ecological niches has the potential to optimize nutrient use.

KEYWORDS: phosphorus budget, integrated aquaculture, *Astyanax lacustris*, *Macrobrachium rosembergii*, *Prochilodus lineatus*.

1. INTRODUCTION

The production of aquatic organisms in earthen ponds presents the greatest contribution to food security in aquaculture and involves several ecological compartments, which accumulate nutrients and are linked to natural productivity in ponds (David *et al*., 2017a; Flickinger *et al* 2020a; FAO, 2020). Therefore, detailed knowledge of the nutrients that pass-through aquaculture ponds is essential to optimize the system and direct their accumulation in the cultivated species (David *et al*., 2017b). The first step in understanding nutrient cycling in aquaculture systems is to quantify some key elements, such as phosphorus, in each compartment (Mariscal-Lagarda and Páez-Osuna 2014; David *et al*., 2017a).

Phosphorus is an essential element for all forms of life on Earth, being present in several metabolic processes and biochemical reactions (Corbridge, 2013a, b; Huang *et al*., 2020). This nutrient is a limiting factor in the production of aquatic and terrestrial organisms. In fish farming, it is intentionally introduced as fertilizers containing calcium or ammonium phosphate to stimulate the growth of phytoplankton, which serve as food for fish and prawn (Flickinger *et al*., 2020a; Boyd *et al*., 2020). Phosphorus is rapidly absorbed by bottom soils, increasing the need for reapplication of fertilizers to maintain the phytoplankton community (Boyd *et al*., 2020). Another factor for the input and accumulation of this nutrient in the system is the diet offered, due to the dispersion of uneaten feed or because of metabolic products of the cultivated organisms (Amirkolaie, 2011; Wang *et al*., 2012; Osti *et al*., 2020). Previous studies indicate an accumulation of phosphorus at the bottom of ponds in integrated cultures and monocultures of fish and prawn, ranging from approximately 50 to 70% (Masuda and Boyd, 1994, David *et al*., 2017a; Flickinger *et al*., 2020a).

The accumulated phosphorus becomes available again with rain in empty ponds or by resuspension of particles in management processes. (Masuda and Boyd, 1994; Boyd, 2020). In addition, earth ponds can assimilate and transform nutrients through the mineralization of organic matter, releasing nutrients for organisms in the aquatic food chain. (Boyd, 2020; Flickinger *et al*., 2020b). The phosphorus that accumulates in pond systems can be assimilated by another species that has a complementary ecosystem function in relation to the target species (Marques *et al*., 2016). Therefore, integrating benthic species into traditional fish farming may be more advantageous than monoculture to recover phosphorus accumulation in bottom soils (David, *et al*. 2017a; Flickinger *et al*., 2020a). This production strategy may be is more efficient if the bottom species depend on fish residues and the natural fauna of the ponds to obtain nutrients.

Macrobrachium rosenbergii feeds mainly on detritus and benthic organisms (New and Valenti, 2000), presenting potential for inclusion in integrated farming to optimize the use of materials and energy generated by fish in the water column. The culture of this species is an important economic activity in several countries around the world, mainly in Asia. In America, this activity has local importance in some countries, such as the United States and Brazil (Ming, 2014; Banu and Christiano, 2016; David, *et al*., 2018). *Prochilodus lineatus* feeds on organic debris, benthic fauna and feed and has ecological and environmental importance in aquatic food webs. This allows their integration into polyculture systems (Castagnolli, 1992; Murgas *et al*., 2012; Zuffo *et al*., 2021). This species showed promising results in the integrated culture of tambaqui (*Colossoma macropomum*) and Amazon river prawn (*Macrobrachium amazonicum*) (Franchini *et al*., 2020). Therefore, in an integrated culture with *Astyanax lacustris*, which swims actively in the water column and has opportunistic omnivorous eating habits, curimbatá and prawn can be an important ally for the recovery of phosphorus at the pond bottom. Some studies provided the phosphorus budget in integrated systems with fish and prawn (Nhan *et al*. 2008; Sahu *et al*., 2015; David *et al*., 2017a; Flickinger *et al*., 2020a). None of them tested the addition of more than one benthic species to the culture. Thus, the objective of this study was to map the phosphorus content in the main ecological compartments of the earthen ponds of the integrated cultures of *A. lacustris*, *M. rosenbergii* and *P. lineatus* in comparison with *A. lacustris*, *M. rosenbergii,* during the 2 months of culture. The systems were compared to each other by measuring the phosphorus content in each compartment. In addition, phosphorus budgets were made for the two culture systems.

2. MATERIAL AND METHODS

2.1. Experimental design

An experiment of integrated cultures was carried out at the Crustacean Sector of the Aquaculture Center, São Paulo State University, Brazil (21º15’22’’S e 48º18’48’’O). The experiment was conduct from January 14 to March 16, 2020, which is in the warm and rainy season. Twelve earthen ponds with area of ~0.015 ha and depth of 1 m were used. The sediment accumulated in previous experiments was not removed. However, the ponds were drained, air-dried, and supplied with hypereutrophic water (Flickinger *et al*., 2019). It has been used in cultures without renewal with success (Kimpara *et al*., 2011; David *et al*., 2017a, b). Throughout the experimental period, the ponds were maintained without water renewal, and the supply water was used only to replace the evaporation and seepage. The experimental design was completely randomized, consisting of two treatments and six replications. The treatments were integrated culture of yellow tail lambari (*Astyanax lacustris*) and giant river prawn (*Macrobrachium rosenbergii*) (LP) and integrated culture of yellow tail lambari, giant river prawn and curimbatá (*Prochilodus lineatus*) (LPC). The stocking density of the animals was lambaris = 40 ind.m^{-2}, prawn = 5 ind.m^{-2} and curimbatás = 3 ind.m^{-2}. The experiment lasted 62 days. This was the time required for lambari reach the size of 7 cm to be marketed as live bait. Prawn was marketed as human consumption and curimbatá was marketed as restocking.

2.2. Monitoring of water variables

Some physical and chemical variables waters were monitored daily at 8:00 a.m. and 4:00 p.m. with a YSI 556 Professional Plus multiparameter probe. Data are showed for the morning and afternoon periods, respectively, as mean ± standard deviation of the mean among all replicates of the treatments in the 12 ponds (table 1).

Table 1. Chemical variables water for the two farming systems. LP = integrated culture of lambari and prawn; LPC = integrated culture of lambari, prawn and curimbatá; *P* = probability to obtain the value of the *t*-statistic if the means are not different.DO: Dissolved Oxygen; Cond: electrical conductivity.

Chemical Variables Water	**LP***	**LPC***	***P***
	Morning		
Temperature (°C)	27.7 ± 0.2	27.6 ± 0.1	0.312
DO (mg L^{-1})	6.1 ± 0.6	6.3 ± 0.3	0.584
Cond. (µS cm^{-1})	117 ± 0.7	117 ± 1.1	0.989
pH	8.8 ± 0.4	8.7 ± 0.2	0.318
	Afternoon		
Temperature (°C)	30.7 ± 0.2	30.6 ± 0.2	0.303
DO (mg L^{-1})	11.3 ± 0.5 ^{a}	12.4 ± 0.7 ^{b}	0.041
Cond. (µS cm^{-1})	122 ± 5.2	121 ± 0,8	0.624
pH	9.6 ± 0.1	9.6 ± 0.1	0.773

* Values followed by the same letter in a line did not differ statistically. Data are presented as mean ± standard deviation of the mean.

2.3. Diet

The target species of the production was the yellow tail lambari, which was fed twice a day with 10% of its biomass. The diet provided was commercial feed for omnivorous fish (Guabi-Aqua Onívoros QS, Guabi Tech Juvenil – 36% crude protein, 2.3 mm granulometry). This diet is currently offered by Brazilian lambari producers because there is no commercial feed formulated for the species. Curimbatá and giant river prawn benefited from the nutrients, by-products and residues of the diet supplied to lambari.

2.4. Stocking, biometric data and harvest

Before stocking, the animals were acclimated using the same method used by farmers. The ponds were populated with lambari fingerlings aged 15 days, prawn post-larvae aged 20 days and curimbatás aged 1 year. The initial mean length (cm) and mass (g) ± standard deviations were respectively, *A. lacustris* = 1.5 ± 0.2 cm and 0.04 ± 0.02 g, *M. rosenbergii* = 1.8 ± 0.4 cm and 0.05 ± 0.03 g, and *P. lineatus* = 13.4 ± 1.3 cm and 27 ± 9.9 g. Random samples of 100 individuals per species were collected to perform biometrics during the experiment. The individual animal mass was obtained using an electronic balance (Marte - AS 2000C, Brazil) with an accuracy of 0.01 g. The total length of *M. rosenbergii* and the total and standard length of the fish were determined, respectively with a 0.01 mm precision digital caliper and a 0.1 cm precision wooden ichthyometer. The total length of *M. rosenbergii* was determined by the distance from the distal margin of the rostrum to the distal end of the telson (Moraes-Riodades and Valenti, 2002). The total length of the fish corresponded to the distance between the mouth and the end of the longest lobe of the caudal fin, while the standard length referred to the distance between the mouth and the end of the caudal peduncle (Holden and Raitt, 1974). At the end of the experiment, all the ponds were dried and harvested. The surviving fish and prawn were counted and a random sample of 100 individuals of each species from each pond was weighed on an electronic balance (Marte - AS 2000C; precision 0.1g) and the mean value for each species was calculated.

2.5. Sample collection for analysis of phosphorus

Sample collections were performed fortnightly over 60 days. Samples were obtained from the various ecological compartments of each pond as described below.

2.5.1. Water

Samples of supplied water and the water drained during harvests were collected in all ponds in both treatments. To estimate the seepage and thus measure the amount of replacement every two weeks, the pond inlet water was closed for a period of eight hours (8:00 a.m. - 4:00 p.m.). Based on the difference in water level and pond area, the volume lost by seepage was calculated. With this data, was estimated the amount of water that entered the pond for fifteen days to maintain its level stable. The phosphorus load of the inlet, outlet and seepage water was calculated multiplying by phosphorus concentration in water by total volume of each pond. Phosphorus concentrations were determined using the persulfate method (APHA, 2017, 4005-P B5) and the stannous chloride method (APHA, 2017, 4500-P D) using a mass spectrophotometer (ASC-5 and UV-1000, Shimadzu).

2.5.2. Animals, sediment, and diet

Samples of 5 g of each species by pond were collected in the stocking and harvesting. This is the quantity required to have a representative sample and in sufficient quantity to perform the required analyzes. The collected animals were slaughtered on ice and stored in properly identified plastic bags. For sediment collection in the pond, tripton collectors were used. These collectors are made up of six 1.766 L PVC pipes, measuring 9.7 cm in diameter and 25.4 cm in length. The collectors were filled with salted water, placed inside each pond and kept throughout the culture. Then, the sedimented material was removed from each PVC pipe, mixed, and transferred to polyethylene bottles. Samples of the diet were also collected. All sampled material was dried in a forced air oven (Nova Ética - 700-7DE) at 60˚C until reaching a constant mass. The samples were ground using compatible mills. These were stored in falcon tubes and properly identified. For determination of phosphorus content, metavanadate colorimetry method (according to Michelsen, 1957) was conducted using a mass spectrophotometer (ASC-5 and UV-1000, Shimadzu) (AOAC, 1995; 969.31 A). The input of phosphorus through feed was calculated by multiplying the phosphorus concentration measured in the diet by the total amount of diet supplied. The input and output of phosphorus in animals was calculated by multiplying phosphorus concentration in the body of animals from each species and total biomass stocked and harvested of each species; then the values of all species were summed.

2.6. Phosphorus budget calculation

Phosphorus contents in all the inputs compartments were summed. Then, the total carbon outputs (TO) were subtracted from the total input (TI). The result corresponds to the unaccounted portion (UP). The equations used were:

a) $TI = IW + SF + SP + D$
b) $TO = WO + SW + HF + HP + S$
c) $UP = TO - TI$

in which, IW (inlet water), SF (stocked fish), SP (stocked prawn) and D (diet) refer to the content of phosphorus at the input compartments; and WO (water outlet), SW (seepage water), HF (harvested fish), HP (harvested prawn) and S (sediment) refer to the phosphorus content at the outlet compartments.

2.7. Statistical analysis

All variables obtained were subjected to normality and homoscedasticity analyses through the Shapiro-Wilk and Bartlett tests, respectively. Data did not deviate from normality and homecedascity, and thus, they were subjected to statistical analysis using the t-test to verify the differences in the means between both treatments. Statistical analyses were carried out in R software (version 4.0.3), and the level of significance considered was $\alpha = 0.05$.

3. RESULTS

No statistical differences were obtained in total yield and Feed Conversion Ratio (FCR) between treatments. In addition, it is noted that the same amount of feed provided in the culture of the two species was sufficient for the culture of three species (Table 2). No statistically significant difference between treatments was obtained mean final mass, mean final length, survival, and yield of lambari. Therefore, the lambari presented similar growth and survival in both culture systems. Prawn survival did not differ between treatments. However, there was a difference in the mean final length, mean final mass and yield, being significantly higher in the LP treatment.

Table 2. Mass, length, survival, yield, and Feed Conversion Ratio (FCR) obtained in each treatment. LP = integrated culture of lambari and prawn, LPC = integrated culture of lambari, prawn and curimbatá. *P* = probability to obtain the value of the *t*-statistic if the means are not different. Initial size: *A. lacustris* = 1.5 ± 0.2 cm; 0.04 ± 0.02 g; *M. rosenbergii* = 1.76 ± 0.44 cm; 0.05 ± 0.03 g; *P. lineatus* = 13.4 ± 1.3 cm; 27 ± 9.9 g.

	LP*	**LPC***	***P***
Total yield (t.ha^{-1})	1.9 ± 0.5	2.5 ± 0.3	0.092
Total diet supplied (kg/ha)	2.5 ± 0.8	2.4 ± 0.3	0.839
FCR	1.3 ± 0.5	1.5 ± 0.3	0.457
A. lacustris			
Mean final mass (g)	5.6 ± 1.9	6.2 ± 2.2	0.761
Mean final length (cm)	6.5 ± 0.9	6.8 ± 0.9	0.763
Survival (%)	70	62	-
Yield (t.ha^{-1})	1.5 ± 0.5	1.5 ± 0.4	0.861
M. rosenbergii			
Mean final mass (g)	8.9 ± 0.9 ^{a}	5.9 ± 1.2 ^{b}	0.007
Mean final length (cm)	9.8 ± 0.5 ^{a}	8.5 ± 0.6 ^{b}	0.008
Survival (%)	89	89	-
Yield (t.ha^{-1})	0.4 ± 0.03 ^{a}	0.3 ± 0.05 ^{b}	0.003
P. lineatus			
Mean final mass (g)	-	33.9 ± 4	-
Mean final length (cm)	-	13.6 ± 1.3	-
Survival (%)	-	75	-
Yield (t.ha^{-1})	-	0.8 ± 0.2	-

* Values followed by the same letter in a line did not differ statistically. Data are presented as mean ± standard deviation of the mean.

There were no statistically significant differences between the two treatments analyzed in all compartments ($P < 0.05$) (Table 3). The phosphorus input in the LPC was greater than in the LP treatment, due to the addition of curimbatá. Respectively in the LP and LPC treatments, the diet represented approximately 86.1 and 75%, the inlet water represented approximately 13.6 and 15.3% and stocked fish and prawn represented approximately 0.23 and 9.8% of total phosphorus inputs (Figs. 1-2). Therefore, phosphorus input into the system occurred mainly through the diet in both treatments. The largest portion of phosphorus that left the system was accumulated in the sediment, representing approximately 55.9 and 57.6%. The remaining phosphorus accumulated in the biomass of harvested fish and prawn, representing approximately 30.3 and 29.4%, in the seepage water, corresponding to 12.2 and 11.8% and in the water released into the effluent, representing approximately 1.6% and 1.2%. (Table 4). The P:N ratio was approximately 1:4 in the water compartments and 1:1 in the sediment. In both treatments, normal amounts of these nutrients were maintained (Table 5).

Unaccounted phosphorus for in the LP treatment was approximately 2.9% relative to the system input. Unaccounted phosphorus for in the LPC treatment was approximately 22.2% in the systems outlet compartments. The assimilation of phosphorus in the biomass of the cultivated species was similar in both treatments. In the LP and LPC treatments, respectively, the harvested animals incorporated 33.9 and 32.4% of the phosphorus contained in the supplied diet and 29.2 and 24.3% of the total phosphorus input (Table 4).

Table 3. Phosphorus content in each ecological compartment for the two farming systems. LP = integrated culture of lambari and prawn. LPC = integrated culture of lambari, prawn and curimbatá. *P* = probability to obtain the value of the *t*-statistic if the means are not different.

Ecological compartments (kg P ha^{-1})	**Treatments***		***P***
	LP	**LPC**	
Input			
Diet	149 ± 49.9	149.2 ± 18.6	0.992
Inlet water	23.6 ± 7.5	30.4 ± 11.5	0.302
Stocked lambari	0.4 ± 0.1	0.4 ± 0.04	0.874
Stocked prawn	0.04 ± 0.01	0.04 ± 0.004	0.874
Stocked curimbatá	-	19 ± 1.9	-
Total	172.9 ± 51.1	199 ± 22.2	0.368
Output			
Water outlet	2.7 ± 0.3	2.7 ± 0.4	0.879
seepage water	20.5 ± 7.5	27.3 ± 11.2	0.292
Harvested lambari	45.1 ± 22.8	44.9 ± 17.4	0.991
Harvested prawn	5.8 ± 1.9	3.9 ± 0.9	0.090
Harvested curimbatá	-	19.2 ± 3.1	-
Sediment	93.9 ± 18.3	133.3 ± 34.2	0.108
Total	168 ± 28.1	231.3 ± 44.3	0.055
Unaccounted			
Input – Output	5 ± 38.2	-51.3 ± 58.4	0.114

* Data are presented as mean ± standard deviation of the mean.

Table 4. Phosphorus incorporated by animals based on the overall diet supplied and total phosphorus input throughout the culture cycle. LP = integrated culture of lambari and prawn. LPC = integrated culture of lambari, prawn and curimbatá. *P* = probability to obtain the value of the *t*-statistic if the means are not different.

Phosphorus incorporated by animals (%)	**Treatments**	
	LP	**LPC**
Diet-Phosphorus		
Lambari	30	29.8
Prawn	3.9	2.6
Curimbatá	-	0
Total	33.9	32.4
Total input of Phosphorus		
Lambari	25.9	22.4
Prawn	3.3	1.9
Curimbatá	-	0
Total	29.2	24.3

Table 5. Proportion of compartments of phosphorus and nitrogen in the culture cycle. LP = integrated culture of lambari and prawn. LPC = integrated culture of lambari, prawn and curimbatá. P = phosphorus, N = nitrogen.

P:N ratio	**LP treatment**		**LPC treatment**	
	Proportion	**Value**	**Proportion**	**Value**
Water inlet	1:4	0.2 ± 0.02	1:4	0.3 ± 0.03
Seepage water	1:4	0.3 ± 0.02	1:4	0.3 ± 0.04
Outlet water	1:4	0.3 ± 0.1	1:4	0.3 ± 0.1
Sediment	1:1	1 ± 0.5	1:1	0.9 ± 0.4

* Data are presented as mean ± standard deviation of the mean.

Figure 1. Phosphorus budget in integrated culture of *Astyanax lacustris* and *Macrobrachium rosenbergii* (LP). Values are shown in percentages based on the total input and output to pond.

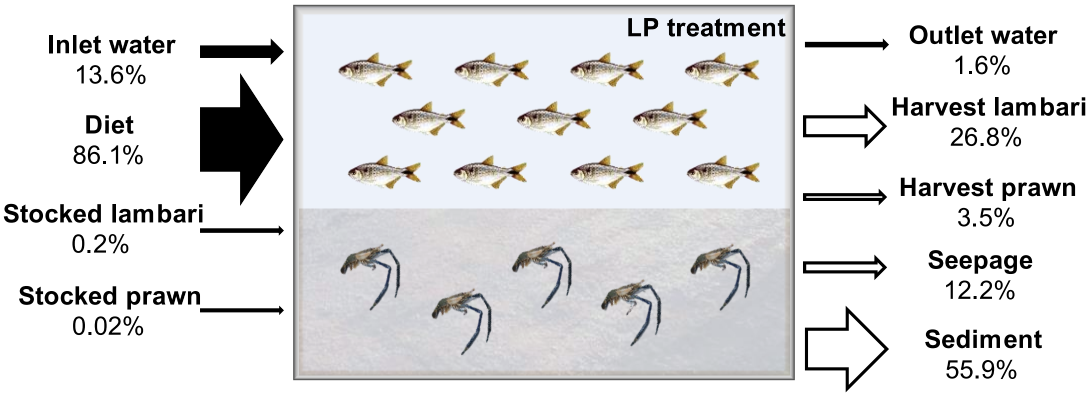
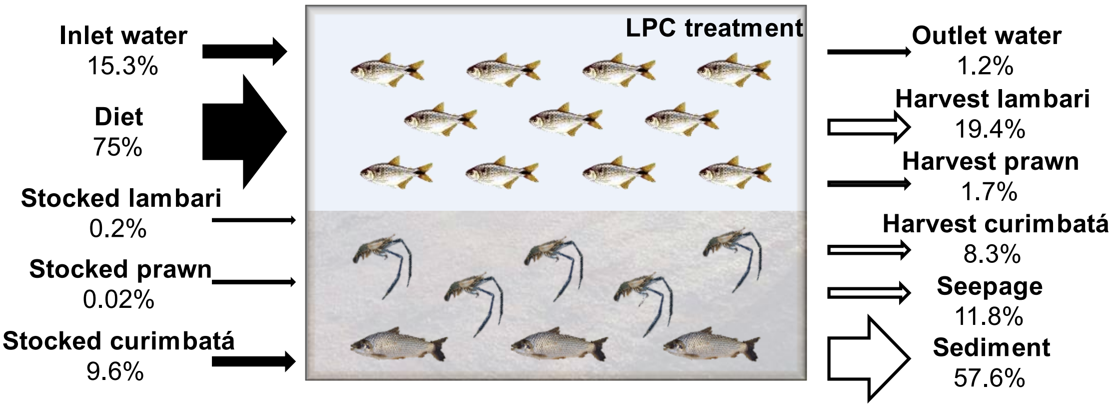


Figure 2. Phosphorus budget in integrated culture of *Astyanax lacustris*, *Macrobrachium rosenbergii* and *Prochilodus lineatus*. Values are shown in percentages based on the total input and output to pond.



4. DISCUSSION

The integrated aquaculture systems composed by yellow tail lambari and giant river prawn (LP), and yellow tail lambari, giant river prawn and curimbatá (LPC) have similar resources available inside ponds and received the same quantity of diet. However, curimbatá did not gain mass and prawns showed a lower growth in the presence of curimbatá. These results suggest a competition of curimbatá and prawn by resources, mainly food. In previous studies in the integrated culture of yellow tail lambari, Amazon river prawn (*Macrobrachium amazonicum*) and curimbatá, the fish developed well and grow were not affected by the two bottom species and total yield increased (Marques *et al*., 2021). The same were observed in the integrated culture of tambaqui (*Colossoma macropomum*), Amazon river prawn and curimbatá (Franchini *et al*., 2020). The curimbatás used in both studies mentioned above were fingerlings of approximately 2.6 g (Franchini *et al*., 2020) and 2.5 g (Marques *et al*., 2021), while the curimbatás of the present study were juveniles of ~ 27 g, which were maintained in high densities for ~ 1 year. A compensatory growth in curimbatás was expected but it did not come through.

Compensatory growth is a phase of accelerated growth when favorable conditions are restored after a period of growth depression (Ali *et al*., 2003). Compensatory growth can also be defined as any growth that reduces variance in the system (Atchley, 1984) or as the negative correlation between the growth of a trait at successive time intervals (Ricker, 1969, 1975; Riska *et al*., 1984). This mechanism is important for fisheries management, aquaculture, and life history analysis because it can compensate for the effects of growth disruptions (Ali *et al*., 2003). Compensatory growth may not have occurred in the experiment due to the short culture period of approximately ~2 months, which is the developmental period of the yellow tail lambari.

No significant difference was observed in the phosphorus compartment contents between the two IMTA systems. The high variability of the data decreased the power of the *t*-test (Sokal and Rohlf 1995), which made it more difficult to detect differences between compartments. Phosphorus entered the system mainly through the diet (86.1% in LP and 75% in LPC), inlet water (13.6% in LP and 15.3% in LPC) and stocked curimbatás (9.6% in LPC). These data corroborated those found by Van Khoi and Fotedar (2010), Sahu *et al*. (2015), David *et al*. (2017a) and Flickinger *et al*. (2020a), in whose studies the diet was also the main source of phosphorus in the system. Most of the phosphorus that inputs the system accumulated on the pond bottom as sediment (55.9% in LP and 57.6% in LPC) and in fish biomass (30.3% in LP and 29.4% in LPC). This mapping indicates that phosphorus was used inefficiently due to the large amount of phosphorus lost to the sediment. However, the current results are superior to previous studies of integrated culture of tilapia and Amazon river prawn, in which ~60-68% of phosphorus accumulated on the bottom of the pond as sediment and only ~18-26% of the total output phosphorus was in the fish plus prawns biomasses (David *et al*., 2017a); and monocultures and integrated cultures of tambaqui and Amazon river prawn with ~31–73% sediment accumulation and only ~7-14% in fish biomass (Flickinger *et al*., 2020a). The remaining significant load of phosphorus in the present study was lost by seepage, corresponding to (12.2% in LP) and (11.8% in LPC).

The integrated culture of yellow tail lambari with prawn had a total yield 1.9 t/ha/cycle and recovered 50.9 ± 23.7 kg of P/ha/cycle, that is, 26.8 ± 12.5 kg of phosphorus for each tonne produced. In the same integrated culture with the addition of curimbatá, 2.5 t/ha/cycle of total yield were obtained, and 67.9 ± 19.9 kg of P/ha/cycle were recovered, that is, 27.1 ± 8 kg of phosphorus for each tonne produced. The integrated culture with tilapia and Amazon river prawn recovered 5.3 kg of P/t produced (David *et al*., 2017a) and the integrated culture with tambaqui and Amazon river prawn recovered 2.5 kg of P/t produced (Flickinger *et al*., 2020a). In this present work, both treatments had the same proportion of diet offered, and in both cases, animals incorporated ~1/3 of the diet offered. However, the prawn accumulated less phosphorus in their biomass due to the reduction in their growth in the presence of curimbatá. Studying the agonistic behavior of prawn seems to be the logical first step in understanding this reduction, since all growth control mechanisms involve an aggressive behavioral component (Karplus, 2005). Thus, in this experiment the decrease in growth rate may be due to direct competition for food, space, and the increase in energy expenditure in motor activity because de presence of the curimbatá. Future adaptations to the density of both species should be made to reduce this competition.

In this study, prawn and curimbatá fed on bottom fauna, fish feces and uneaten lambari diet, as stated by New and Valenti (2017) for integrated culture systems with tilapia. Diet corresponded to ~75-86.1% of the phosphorus inputs, which is higher than it was previously obtained in the culture of tilapia with Amazon river prawn (50 – 61%) (David *et al*., 2017a) and the culture of tambaqui and Amazon river prawn (63-76%)

(Flickinger *et al*., 2020a). The results indicate that diet supply is the main driver that affects the phosphorus content in different compartments in the ponds. The supplied diet probably increased the organic phosphorus in the water column, which consumes oxygen in the decomposition processes. Sedimentation is compensated by daily feeding (David *et al*., 2017a; Flickinger *et al*., 2020a). The process of phosphorus exchange between the water column and the sediments plays a smaller role than feeding (David *et al*., 2017a). Furthermore, it is a daily input into the system, with high values at the end of the production cycle (Boyd *et al*., 2020). This process is more intense at the bottom of the pond, which can make the pond soil a source of phosphorus because of conditions that release metal-bound phosphates into the water column (Flickinger *et al*., 2020a; Boyd, 2020).

The inlet water compartment was the second largest input of phosphorus for the two integrated cultures, representing ~13.6-15.3%. Previous studies carried out on the same aquaculture farm reported higher values. David *et al*. (2017a), obtained values of 17-27% for the integrated culture of prawn and tilapia; Flickinger *et al*. (2020a), obtained values of 21-37% for tambaqui and prawn culture. Inlet water values for other mass budgets ranged from 0.3% (Sahu *et al*., 2013a; Adhikari *et al*., 2014) to 38% (Casillas-Hernández *et al*., 2006). This large variation results from the volume of water used, which is specific to each location and the level of phosphorus in the inlet water source. The phosphorus contribution observed in the present study is due to the use of nutrient-rich water from a reservoir that receives effluents from previous cultures (Flickinger *et al*., 2019, 2020a, b; Dantas *et al*., 2020; Franchini *et al*., 2020; David *et al*., 2017a, b, 2021) combined with the large volume used to replace the seepage and evaporation. The reuse of nutrient-rich water has been suggested as an alternative source of phosphorus for target organisms (Kimpara *et al*., 2011). Enhancing natural productivity can recover dissolved nutrients converting them in an ingestible form to fish and prawns. Nevertheless, the effectiveness of using phosphorus in nutrient-rich recycled water is uncertain because of its insolubility (Boyd, 1995; Masuda and Boyd, 1994a, b), which limits its availability for uptake into the aquatic food chain. (Boyd, 2020).

Leaving phosphorus from the system by water outlet during harvest is ~1.2-1.6%. These proportions were lower than those of entering phosphorus by inlet water in all treatments. Most of the water carries dissolved phosphorus by seepage in the pond bottoms, which can carry phosphorus to the soil. This retention is associated with sedimentation and decomposition processes (Boyd, 2020). Similar results were observed in tambaqui and prawn cultures (Flickinger *et al*., 2020a). The sediment has favorable characteristics for phosphorus retention. In the present study ~55.9-57.6% of phosphorus inputs accumulated as sediment. Sedimentation increased with the addition of curimbatá. The combined bioturbation of prawn and curimbatá at the bottom of the ponds resuspends the sediment, making phosphorus, nitrogen, and other nutrients available in the water column (Adámek and Maršálek, 2013; Boyd, 2020). The re-availability of nutrients is important for optimizing the use of resources, and consequently increasing sustainability.

Nitrogen and phosphorus are essential nutrients that regulate aquatic productivity, varying in quantities and proportions between species (Boyd, 2020). Therefore, it is essential to maintain a balanced P:N ratio to ensure healthy growth of aquatic organisms, minimize nutrient waste and reduce environmental load. The ratio of these nutrients to water and organic matter can vary from 1:1 to 1:7 in normal scenarios. Excessive amounts of phosphorus compared to nitrogen can cause eutrophication of ponds, reducing the level of oxygen. On the other hand, low amounts of phosphorus in relation to nitrogen can affect the growth and development of cultivated aquatic organisms (Boyd, 2020). In this experiment, the amounts of these nutrients were approximately 1:4 in the water compartments and 1:1 in the sediment. Thus, in both treatments, adequate amounts of these nutrients were maintained, facilitating mineralization and/or immobilization processes. Future studies may corroborate the hypothesis that the system acts as a filter, reducing pollution in this production system.

Unaccounted portions are common in mass budget reviews. Several studies have reported rates from 3.6 to 50% (Van Khoi and Fotedar, 2010; Le Van and Fotedar, 2011; Sahu *et al*., 2013a, b; Adhikari *et al*., 2014; David *et al*., 2017a; Flickinger *et al*., 2020a). In the present study, unaccounted phosphorus in the LP was 5 ± 38.2 kg/ha in the input compartments, that is, 2.9%. In the LPC treatment it was 51.3 ± 58, 4 kg/ha, that is, 22.2%, but in the output compartments. Identifying unquantified phosphorus outputs extrapolated from other nutrient budgets is difficult to determine (Flickinger *et al*., 2020). This may be associated with input of nutrients by runoff, fallen leaves and flowers, trees that may be close to the pond, and waterfowl droppings. The accumulation of organic material in the bottom sediments certainly led to acidic and anoxic conditions, which could go deeper into the soil with seepage (Boyd, 2020). In addition, the phosphorus levels in the soil were not quantified before the experiment, which could increase the total phosphorus production and, consequently, a portion unaccounted for in the phosphorus input of the LPC treatment.

5. CONCLUSION

The present work helped to understand the phosphorus budget in earthen ponds. The systems were efficient in removing phosphorus from the inlet water and accumulating phosphorus from the diet inside the ponds or in the target species produced. The long period of water retention inside the ponds allowed sedimentation and nutrient cycling. Both treatments have a great capacity for recovering phosphorus in produced biomass. In the integrated culture of lambari and prawn, around 26.8 kg of phosphorus was assimilated for each tonne produced. The addition of curimbatá was not effective to increase the assimilation, which was 27.1 ± 8 kg of phosphorus per tonne produced. The addition of curimbatá reduced prawn growth. This loss of performance may be directly linked to the competition between prawn and curimbatás that occurred at the bottom of the ponds. The performance of species at certain densities is still uncertain since some species do not have a specific settlement design. Carrying capacity can help with this process, filling some of the gaps left by the nutrient budget. Future research should also focus on the use of phosphorus accumulated in the pond bottoms, which may contribute to other types of culture.

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CAPÍTULO 3

NITROGEN BUDGET OF *Astyanax lacustris* FARMING IN INTEGRATED CULTURE WITH *Macrobrachium rosenbergii* AND WITH *Macrobrachium rosenbergii* AND *Prochilodus lineatus*

ABSTRACT

In this study the hypothesis tested was that the addition of benthic species with complementary ecological niches can optimize the use of nitrogen in earthen ponds. For this, an experiment was developed in twelve ponds, with two treatments and six repetitions. Each treatment corresponds to a production system, i.e., integrated cultures of yellow tail lambari (Astyanax lacustris) and giant river prawn (*Macrobrachium rosenbergii*) (LP) in comparison with yellow tail lambari, giant river prawn and curimbatá (*Prochilodus lineatus*) (LPC). Animals, water, diet, sediment, and gas were collected throughout the experiment to quantify the nitrogen inputs and outputs of the system. Respectively, in the LP and LPC treatments, the largest amount of nitrogen input the system mainly through the supplied diet ~56.4 and 46.4%, followed by inlet water ~43.4 and 45%. Respectively, in the LP and LPC treatments, the harvested fish and prawn represented approximately 11.1 and 11.9% of the nitrogen output, the seepage represented 16.2 and 17.6%, water outlet represented approximately 2.2 and 1.8%, N2 released by bubbles represented approximately 46.3 and 40.3%, gases released by diffusion represented approximately 0.1 and 0.04% and the sediment represented approximately 23.9 and 27.9%. Overall, this mapping indicates that the two integrated cultures in this study were effective in converting diet nitrogen into harvested biomass, with values of ~42.8-40%. All systems were efficient in removing nitrogen from the water. The long period of water retention in the ponds allowed sedimentation, decomposition, and denitrification processes. Thus, the nitrogen was rapidly transformed through the aquatic nitrogen cycle and released into the atmosphere as molecular nitrogen gas. Therefore, both systems demonstrated great potential for optimization in the use of resources.

KEYWORDS: nitrogen budget, integrated aquaculture, *Astyanax lacustris*, *Macrobrachium rosembergii*, *Prochilodus lineatus*.

1. INTRODUCTION

In 2020, fishing and aquaculture production reached a historic record of 214 million tons, worth around US$424 billion. Aquatic animal production in 2020 was 60% higher than the 1990s average (FAO, 2022). The growing global demand and the industrialization of the activity can increase the number of unused resources, gas emissions and eutrophication of aquatic environments, mainly from the expansion and intensification of fed aquaculture systems (Clark and Tilman, 2017; Flickinger *et al*., 2020a). Feed accounts for most of the allochthonous nitrogen added in ponds of fish, but only 25% to 30% of feed nitrogen is recovered as harvested biomass (Boyd and Tucker, 1995, 2012). The remaining nitrogen is excreted as ammonia and organic nitrogen or accumulates as solid material from feces and uneaten food (Walsh and Wright, 1995).

Nitrogen is a key element because it is part of the constitutive proteins and enzymes of all organisms. This nutrient is essential in any animal's diet. Diets must be suitable for each species to provide the suitable combination of amino acids. Content of proteins in excess or a lack of some amino acids cause greater loss of nitrogen in the water (Boyd, 2020; Marques *et al*., 2021). Understanding the nitrogen budget is a prerequisite for achieving waste reduction (Mariscal-Lagarda and Paez-Osuna, 2014), decreasing the use of chemical fertilizers, and mainly optimizing the use of commercial diets in aquaculture farms. Thus, managing the cycle of this nutrient in water has the potential to increase the sustainability of aquaculture (Flickinger *et al*., 2019), which can reduce waste and help with food management (Marques *et al*., 2016; David *et al*., 2017a).

In this context, the integration of typical species from different trophic levels has been suggested to increase sustainability when compared to traditional monoculture (Chopin, 2014). Nitrogen accumulated in monoculture systems can be assimilated by a species that has a complementary ecosystem function in relation to the target species (Marques *et al*., 2016). Previous studies have shown the occurrence of high amounts of nutrients accumulated on the bottom of ponds at the end of integrated fish and prawn farming (Flickinger *et al*., 2019, 2020a, 2020b; David *et al*., 2017a, 2017b, 2021). This suggests that these systems can feed more iliophagous species in the same ponds. The inclusion of curimbatá (Prochilodus lineatus) in the integrated culture of tambaquis (*Colossoma macropomum*) and Amazon river prawn (*Macrobrachium amazonicum*) showed very promising results (Franchini *et al*., 2020). This species feeds on organic detritus, benthic fauna, and feed (Castagnolli, 1992). Curimbatá has an important representation in national aquaculture and can be commercialized both for human consumption and for basin repopulation initiatives in southeastern Brazil (Valenti *et al*. 2021).

Therefore, the objective of this work is to obtain information about the nitrogen budget and the possible changes in aquaculture production systems, using two fish and a prawn species. The nitrogen budget in the integrated culture of yellow tail lambari (*Astyanax lacustris*) with giant river prawn (*Macrobrachium rosenbergii*) and with giant river prawn and curimbatá was described. Given that previous studies showed that most nutrients accumulate on the bottom of ponds and that, the addition of a prawn species was not sufficient to take advantage of all this material (Flickinger *et al*., 2019, 2020a, 2020b; David *et al*., 2017a, 2017b, 2021), in this work, curimbatá was also added to assess whether there is an increase in the efficiency of assimilation of these nutrients.

2. MATERIAL AND METHODS

2.1. Experimental design

An experiment of integrated cultures was carried out at the Crustacean Sector of the Aquaculture Center, São Paulo State University, Brazil (21º15’22’’S e 48º18’48’’O). The experiment was conduct from January 14 to March 16, 2020, which is in the warm and rainy season. Twelve earthen ponds with area of ~0.015 ha and depth of 1 m were used. The sediment accumulated in previous experiments was not removed. Drainage, drying and replacement of water lost through seepage was carried out according to the method described in Chapter 2. The experimental design was completely randomized, consisting of two treatments and six replications. The treatments were integrated culture of yellow tail lambari (Astyanax lacustris) and giant river prawn (Macrobrachium rosenbergii) (LP), and integrated culture of yellow tail lambari, giant river prawn and curimbatá (Prochilodus lineatus) (LPC). The stocking density of the animals was lambaris = 40 ind.m-2, prawn = 5 ind.m-2 and curimbatás = 3 ind.m-2. The experiment lasted 62 days. This was the time required for lambari to reach the size of 7 cm to be marketed as live bait. Prawn was marketed as human consumption and curimbatá was marketed as restocking species by hydropower companies. The main justification for introducing this last species is the use of nutrient accumulated on the pond bottoms.

2.2. Monitoring of water variables

Temperature, dissolved oxygen, conductivity, and pH were measured daily at 8:00 a.m. and 4:00 p.m. with a YSI 556 Professional Plus multiparameter probe. Data are shown in Chapter 2. All values were suitable for the three farmed species.

2.3. Diet

The target species of the production was the yellow tail lambari, which was fed twice a day with 10% of its biomass. The diet provided was commercial feed for omnivorous fish (Guabi-Aqua Onívoros QS, Guabi Tech Juvenil – 36% crude protein, 2.3 mm granulometry). This diet is currently offered by Brazilian lambari producers because there is no commercial feed formulated for the species. Curimbatá and giant river prawn benefited from the nutrients, by-products and residues of the diet supplied to lambari.

2.4. Stocking and harvest

Stocking, harvest, and biometric procedures were described in Chapter 2. The ponds were populated with lambari fingerlings aged 15 days, prawn post-larvae aged 20 days and curimbatás aged 1 year. At the end of the experiment, all the ponds were dried and harvested. Data on animal size at stocking, yield, survival, and final mean weight of each species at harvest and feed conversion rate are shown in Table 1. These data were discussed in detail in Chapter 2.

Table 1. Mass, length, survival, yield, and Feed Conversion Ratio (FCR) obtained in each treatment. LP = integrated culture of lambari and prawn, LPC = integrated culture of lambari, prawn and curimbatá. *P* = probability to obtain the value of the *t*-statistic if the means are not different. Initial size: *A. lacustris* = 1.5 ± 0.2 cm; 0.04 ± 0.02 g; *M. rosenbergii* = 1.76 ± 0.44 cm; 0.05 ± 0.03 g; *P. lineatus* = 13.4 ± 1.3 cm; 27 ± 9.9 g.

	LP*	**LPC***	***P***
Total yield (t.ha^{-1})	1.9 ± 0.5	2.5 ± 0.3	0.092
Total diet supplied (kg/ha)	2.5 ± 0.8	2.4 ± 0.3	0.839
FCR	1.3 ± 0.5	1.5 ± 0.3	0.457
A. lacustris			
Mean final mass (g)	5.6 ± 1.9	6.2 ± 2.2	0.761
Mean final length (cm)	6.5 ± 0.9	6.8 ± 0.9	0.763
Survival (%)	70	62	-
Yield (t.ha^{-1})	1.5 ± 0.5	1.5 ± 0.4	0.861
M. rosenbergii			
Mean final mass (g)	8.9 ± 0.9 ^{a}	5.9 ± 1.2 ^{b}	0.007
Mean final length (cm)	9.8 ± 0.5 ^{a}	8.5 ± 0.6 ^{b}	0.008
Survival (%)	89	89	-
Yield (t.ha^{-1})	0.4 ± 0.03 ^{a}	0.3 ± 0.05 ^{b}	0.003
P. lineatus			
Mean final mass (g)	-	33.9 ± 4	-
Mean final length (cm)	-	13.6 ± 1.3	-
Survival (%)	-	75	-
Yield (t.ha^{-1})	-	0.8 ± 0.2	-

* Values followed by the same letter in a line did not differ statistically. Data are presented as mean ± standard deviation of the mean.

2.5. Sample collection for analysis of nitrogen

Sample collections were performed fortnightly over 60 days. Samples were obtained from the various ecological compartments of each pond as described below.

2.5.1. Water

Samples of supplied water and the water drained during harvests were collected in all ponds in both treatments. The seepage loss was measure according to the method described in Chapter 2. The water samples were analyzed in a TOC-N - Elementar analyzer to obtain the total nitrogen.

2.5.2. Gases

To determine the absorption and emission of N_2 and N_2O gases by diffusion and boiling (bubbles), five samples in each pond were obtained in the 62 days of the experiment (culture cycle). Diffusive exchange gases with atmosphere in the morning and night and bubbles for during all day samples were obtained. To sample boiling gases, fiberglass funnels were installed on the surface of the ponds and suspended by floats. The funnel has a mouth of 0.3 m, and an angle of 60°, ending in a diameter of 20 mm. At the end of the funnel, a graduated container (600 mL) was connected, which collected the bubbles released during all the culture. For the diffusive collection, day and night samples were obtained. The equilibrium method was used (Matvienko *et al*., 2000), in which an air sample collected near the surface is confined in a diffusion chamber. At the time of installation, a portion of this sample was collected and packaged in glass ampoules (with gel-sealed cap) and another three after 1, 2 and 4 minutes to determine if, after equilibration time, the gas was absorbed by the pond system or released to the atmosphere. All samples collected by transfer tubes were analyzed in the GC-2014 Permanent Gas Analyzer - Shimadzu. Data are obtained in ppm and converted to kg N ha^{-1}. The results refer to the sum of the diffusive and boiling gases occurred during the culture.

2.5.3. Animals, sediment, and diet

Animal and sediment samples of each species by pond were collected in the stocking and harvesting according to the method described in Chapter 2. For determination of nitrogen content, the CHNS Elementary Analyzer - Vario MACRO Cube were used. In this equipment, the samples undergo catalytic combustion, releasing gases that are separated by the retention time in the column. Subsequently, these gases pass through a thermal conductivity sensor (TCD) and are identified by the conductivity difference.

2.6. Nitrogen budget calculation

Nitrogen contents in all the inputs compartments were summed. Then, the total carbon outputs (TO) were subtracted from the total input (TI). The result corresponds to the unaccounted portion (UP). The equations used were:

a) TI = IW + AG + SF + SP + D
b) TO = WO + SW + EG + HF + HP + S
c) UP = TO - TI

in which, IW (inlet water), AG (absorbed gases), SF (stocked fish), SP (stocked prawn) and D (diet) to refer the content of nitrogen at the input compartments; and WO (water outlet), SW (seepage water), EG (emitted gases), HF (harvested fish), HP (harvested prawn) and S (sediment) refer to the nitrogen content at the outlet compartments.

2.7. Statistical analysis

All variables obtained were subjected to normality and homoscedasticity analyses through the Shapiro-Wilk and Bartlett tests, respectively. Data did not deviate from normality and homecedascity, and thus, they were subjected to statistical analysis using the t-test to verify the differences in the means between both treatments. Statistical analyses were carried out in R software (version 4.0.3), and the level of significance considered was $\alpha = 0.05$.

3. RESULTS

Nitrogen input and output varied between compartments, but no statistical difference was observed. (Table 2). The greatest amount of nitrogen inputs the system through the diet and the inlet water (Table 3). The diet represented approximately 56.4 and 46.4% of the total nitrogen inputs in the LP and LPC treatments, respectively. Inlet water represented approximately 43.4 and 45%, absorbed gases approximately 0.04 and 0.1%, and fish and prawn stocked represented approximately 0.17 and 8.6% of the total nitrogen input in the LP and LPC treatments, respectively (Figs. 1-2).

Respectively, in the LP and LPC treatments, the harvested fish and prawn represented approximately 11.1 and 11.9% of the nitrogen output, the seepage represented 16.2 and 17.6%, water outlet represented approximately 2.2 and 1.8%, N_2 released by bubbles represented approximately 46.3 and 40.3%, gases released by diffusion represented approximately 0.1 and 0.04% and the sediment represented approximately 23.9 and 27.9% (Figs. 1-2).

Most of the nitrogen left the ponds through the gases released in the two treatments. Nitrogen gas (N_2) was the major gas released in both treatments, accounting for 46.3 and 40.3% in the LP and LPC treatments, respectively. Most of the gases were dissipated to the atmosphere by the release of bubbles. Diffusion emission was low in both treatments. Nitrogen was significantly higher in seepage water than outlet water in both treatments. The accumulated sediment at the bottom of the ponds used for the LP treatment was smaller than the LPC treatment, not differing from each other. The P:N ratio was approximately 1:4 in the water compartments and 1:1 in the sediment, presenting normal values (Chapter 2) and the C:N ratio to sediment was approximately 11 in LP treatment and 13 in LPC treatment. Unaccounted nitrogen in the input compartments was 54.1% in the LP and 58.1% in the LPC. The assimilation of nitrogen in the biomass of the cultivated species represented, in the LP and LPC treatments, respectively, 42.8 and 40% of the diet offered in the culture and 24.1 and 18.6% of the total nitrogen input (Table 3).

Table 2. Nitrogen content in each ecological compartment for the two farming systems. LP = integrated culture of lambari and prawn. LPC = integrated culture of lambari, prawn and curimbatá. *P* = probability to obtain the value of the *t*-statistic if the means are not different.

Ecological compartments (kg N ha^{-1})	**Treatments***		***P***
	LP	**LPC**	
Input			
Diet	125.4 ± 42	125.6 ± 15.6	0.992
Inlet water	96.5 ± 25.2	121.8 ± 59.1	0.408
N_2 absorbed	0.1 ± 0.2	0.03 ± 0.04	0.342
N_2O absorbed	0.01 ± 0.02	0.1 ± 0.1	0.177
Stocked lambari	0.3 ± 0.1	0.3 ± 0.03	0.874
Stocked prawn	0.07 ± 0.01	0.06 ± 0.01	0.874
Stocked curimbatá	-	23 ± 2.3	-
Total	222.3 ± 49.4	270.8 ± 65	0.283
Output			
Water outlet	10.6 ± 2.9	10.4 ± 2.5	0.920
seepage water	78.5 ± 25.8	104.1 ± 57.4	0.393
N_2 released (bubbles)	224.2 ± 120	238.5 ± 115.5	0.873
N_2O released (bubbles)	1.2 ± 0.9 ^{a}	2.9 ± 1.2 ^{b}	0.043
N_2 released (diffusion)	0.1 ± 0.2	0.2 ± 0.3	0.589
N_2O released (diffusion)	0.2 ± 0.1	0.1 ± 0.1	0.260
Harvested lambari	44.7 ± 26	45 ± 22.8	0.984
Harvested prawn	9.3 ± 4.3	5.6 ± 1.4	0.120
Harvested curimbatá	-	19.9 ± 4.9	-
Sediment	115.9 ± 48.7	165.2 ± 85.5	0.147
Total	484.8 ± 153.5	591.9 ± 136.8	0.398
Unaccounted			
Input – Output	- 262.4 ± 130.2	- 344.1 ± 128.2	0.459

* Values followed by different letters in a line differ statistically. Data are presented as mean ± standard deviation of the mean.

Table 3. Nitrogen incorporated by animals based on the overall diet supplied and total nitrogen input throughout the culture cycle. LP = integrated culture of lambari and prawn. LPC = integrated culture of lambari, prawn and curimbatá. N = nitrogen. *P* = probability to obtain the value of the *t*-statistic if the means are not different.

Nitrogen incorporated by animals (%)	**Treatments**	
	LP	**LPC**
Diet-N		
Lambari	35.4	35.6
Prawn	7.4	4.4
Curimbatá	-	0
Total	42.8	40
Total input of N		
Lambari	20	16.5
Prawn	4.2	2
Curimbatá	-	0
Total	24.1	18.6

Figure 1. Nitrogen budget in integrated culture of *Astyanax lacustris* and *Macrobrachium rosenbergii* (LP). Values are shown in percentages based on the total input and output to pond.

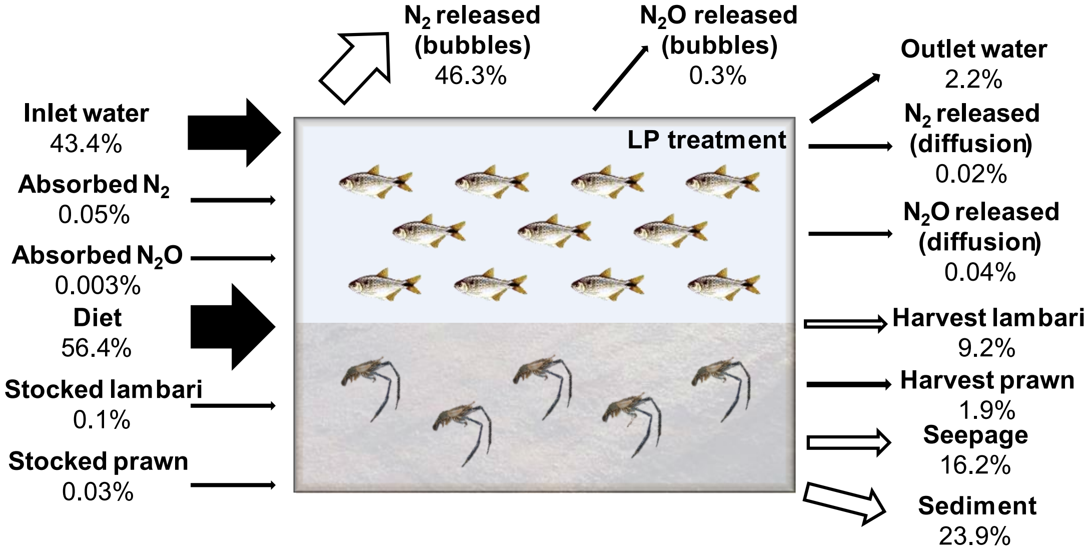
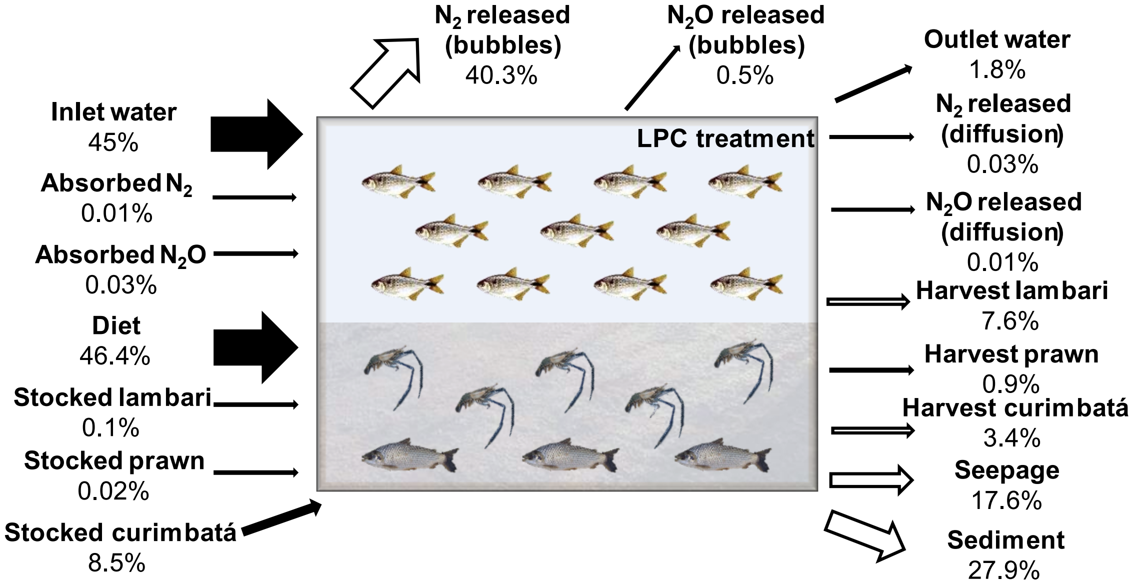


Figure 2. Nitrogen budget in integrated culture of *Astyanax lacustris*, *Macrobrachium rosenbergii* and *Prochilodus lineatus*. Values are shown in percentages based on the total input and output to pond.



4. DISCUSSION

Nitrogen assimilation in the biomass of the lambari, giant river prawn and curimbatá occurred in the two integrated cultures according to the growth of each species in each treatment. The giant river prawn produced in the integrated system with lambari and curimbatá incorporated a smaller amount of nitrogen in the diet offered in relation to the system with yellow tail lambari. The decrease in growth and consequently in nitrogen incorporation may be due to direct competition for food, space, and the increase in energy expenditure in motor activity. Different total yield results were obtained for the integrated culture of yellow-tailed lambari, Amazon river prawn (*Macrobrachium amazonicum*) and curimbatá, showing similar growth when compared to the integrated culture of yellow-tailed lambari and Amazonian river prawn (Marques *et al*., 2021). These results were also observed in the integrated culture of tambaqui (*Colossoma macropomum*) and Amazon river prawn (Dantas *et al*., 2020) in comparison with tambaqui, Amazon river prawn and curimbatá (Franchini *et al*., 2020). The curimbatás used in the studies mentioned above were fingerlings of approximately 2.6 g (Franchini *et al*., 2020) and 2.5 g (Marques *et al*., 2021), while the curimbatás of the present study were juveniles of ~ 27 g and ~ 1 year. These animals were chosen because compensatory growth was expected because they were stocked in high densities for long time. This mechanism is a phase of accelerated growth when favorable conditions are restored after a period of growth depression (Ali *et al*., 2003). Nevertheless, compensatory growth did not occur. This may be due to the short culture period of culture (~2 months). The yellow tail lambari reached the commercial size rapidly, and it was the principal species, the culture should be finished. The comparison between different types of integrated cultures is important for a greater variety of choice for producers, who can choose the most suitable for their farm.

Nitrogen in each ecological compartment showed high variability in the same treatments, evidenced by the high standard deviations. Similar results have been obtained in previous nutrient budget studies (Flickinger, *et al*., 2019, 2020a, 2020b; David *et al*. 2017a, b, 2021). This high variability reduces the reliability of the t-test (Sokal and Rohlf, 1995) and inhibits the detection of some differences between the production systems, as occurred in the gas compartment. The high variability is probably because the experimental units are open-air pond systems with low control of variables and their own dynamics. The development of communities inside the ponds can be different and occur randomly, which alters the dynamics of the system.

Each pond has a different biota that has a great influence on the dynamics of the pond and can increase this variability. At the broadest level, the natural biota of the aquatic system underlies the adaptive diversification of species belonging to different groups, such as bacteria, phytoplankton, zooplankton (Jin *et al*., 2023), benthos. The high variability of the pond compartments possibly contributed to the high portion unaccounted for in the input mass budget, of 54.1% in the LP treatment and 58.1% in the LPC treatment. However, the large amount of uncountable nitrogen dispersed to the atmosphere by bubbles should be investigated. Probably it results from organic nitrogen present in the ponds before starting the experiment.

The largest amount of nitrogen inputs the system mainly through the diet supplied, ~125.4 ± 42 kg/ha, that is, ~56.4% and ~125.6 ± 15.6 kg/ha, that is, ~46.4%. In previous studies, these values ranged from 65% to 97% (David *et al*. 2017a; Zhang *et al*., 2018, 2019; Flickinger *et al*., 2019). The low proportion of feed nitrogen shown here when compared to previous studies is due to the high nitrogen content of the inlet water. In this study, the same amount of diet was offered in both production systems to feed only the yellow tail lambari (target species). The amount of nitrogen incorporated into the fish and prawn biomass was approximately 42.8% for the LP treatment and 40% for the LPC treatment. These values were higher than those obtained in the integrated culture of tilapia and Amazon river prawn (28%; David *et al*., 2017a) and lower than those obtained in the culture of tambaqui and Amazon river prawn (52%; Flickinger *et al*., 2019). The diet ingested by most fish and prawn will change with several behavioral and taxonomic factors (Kulbicki, *et al*., 2005). These factors can directly influence the incorporation of nitrogen by cultivated species, demonstrating the possibility of optimizing the diet offered. Furthermore, the values indicate that the system still allows an optimization in the use of space. This gap can be filled by assessments of the carrying capacity of this system (Cho and Bureau, 1998). Carrying capacity can help optimize resource utilization, reduce negative effects on production, and establish upper environmental limits for aquaculture production (Weitzman and Filgueira, 2020).

The inlet water was the compartment that represented the second highest nitrogen input of approximately 43.4 and 45%, in the LP and LPC treatments, respectively. Previous studies have reported input water contributions to the same facility of approximately 30% for the integrated culture of Amazon river prawn and Nile tilapia (David *et al*., 2017a), 23% and 42% for tambaqui and Amazon river prawn monocultures and 58% for tambaqui and Amazon river prawn integrated culture (Flickinger *et al*., 2019). In studies conducted at other facilities, inlet water nitrogen for the integrated culture of Indian larger carp (*Labeo rohita*) and giant river prawn contributed less than 0.5% of all nitrogen inputs (Sahu *et al*., 2015) and in prawn monoculture 0.5% and 0.6% of all inputs (Adhikari *et al*., 2014; Sahu *et al*., 2013). All experiments were carried out in earth ponds and with the use of fresh water and fertilizers. The high nitrogen in the inlet water of the present study is due to the use of nutrient-rich water from a reservoir that receives aquaculture effluents, used in previous treatments (Flickinger *et al*., 2019), combined with the large volume used to replenish the evaporation and seepage losses. Respectively, the LP and LPC treatments presented nitrogen input values in the water 10 and 13 times higher in relation to those contained in the effluent water. The water leaves the system at harvest, lost to the atmosphere or through seepage into pond bottoms, which can carry nitrogen into the soil. The long period of water retention in the ponds allows sedimentation, decomposition, and denitrification processes (Boyd, 2020). Thus, all systems were efficient in removing nitrogen from the water.

This work had a large portion of the nitrogen input into the system through the diet offered. A portion was incorporated into the animal’s biomass, and another accumulated at the bottom of the ponds. Oxygenated bottom sediments benefit water conditions by oxidizing and detoxifying anaerobic metabolites before diffusing into the water column (Flickinger *et al*. 2019). Previous studies have also shown that conditions in the upper layers of bottom sediments change with increasing population density and swimming activity of Amazon river prawn (Kimpara *et al*., 2013). Sediment is an important source of nitrogen in aquaculture ponds and can remove and store nutrients from the water (Boyd *et al*., 2020). In the LP and LPC treatments, the accumulation of nitrogen in the sediment was approximately 23.9 and 27.9% of the total output load of the systems. These values are similar to those observed in monocultures and integrated cultures of prawn from the Amazon river prawn and tambaquis, which varied between 14% and 42%, of catfish monoculture from 23% to 25% (Gross *et al*., 2000 and in polycultures, in which 24 to 47% were observed (Sahu *et al*., 2015; David *et al*., 2017a). The remaining sediment from the culture accumulated at the bottom of the pond favors decomposition processes, which produce nitrogen mineralization (Boyd, 2020). In low concentration of oxygen, complete or incomplete denitrification processes occur, generating N_2 and N_2O. They both are released into the atmosphere by bubles.

Respectively, in LP and LPC treatments, the removal of nitrogen by bubbles released into the atmosphere after denitrification was greater than the accumulation of nitrogen, showing ~224.2 ± 120 kg/ha, that is, ~46.3% and ~238.5 ± 115.5 kg/ha, that is, ~40.8% of the total system outputs. Furthermore, the data represent values of approximately 79 and 90% higher than those of the diet offered. Systems that lose fewer materials as waste are more efficient and more sustainable (Valenti *et al*., 2018). This indicates that this key element was rapidly transformed through the aquatic nitrogen cycle and released into the atmosphere as molecular nitrogen gas. This probably results from the organic nitrogen present in the ponds before the experiment began. Thus, soil and sediment supported sufficient microbial populations to transform organic matter through the aquatic nitrogen cycle (Flickinger *et al*., 2019). Therefore, the system showed great potential in recycling nitrogen in this compartment, reducing the pollution caused.

Understanding the proportion of carbon to nitrogen (C:N ratio) in organic matter is a key factor in understanding the cycle of these nutrients in ponds and explaining gas emissions. High amounts of nitrogen in organic matter tend to increase the rate of decomposition and the proportion of mineralized organic nitrogen. In contrast, substrates with less nitrogen, the immobilization process occurs, which results in a decrease in the concentrations of ammonia nitrogen and nitrate in the area where decomposition is occurring. In this process, the decomposition of low-nitrogen waste is often greatly accelerated (Boyd, 2020). Previous studies have reported that to function shift of the bacterial community in the sediment should in prawn and fish farming systems with C:N ratio at 12 (Roy *et al*., 2010; Rohmana *et al*., 2015; Perez-Fuentes *et al*., 2016; Zheng *et al*., 2018). In this study, the sediment the C:N ratio was 11 in the LP treatment and 13 in the LPC treatment. When this ratio is above ten, excess carbon can increase aerobic denitrification (Van Niel *et al*., 1993). Aerobic denitrification involves the reduction of nitrate under aerobic conditions by specific microorganisms even in the presence of oxygen (Boyd, 2020). These microorganisms can use oxygen for respiration while simultaneously reducing nitrate. Therefore, this process provides a new avenue for biological modulation of nitrogen, preventing water pollution and allowing efficient nitrate removal in oxygen-rich environments (Seifi and Fazaelipoor, 2012; Ji *et al*., 2015). Overall, in this work the high levels of carbon in relation to nitrogen suggest immobilization of this nutrient in the sediments and alteration of the bacterial community, accelerating its aquatic cycle and causing high releases of N_2 into the atmosphere.

In both treatments the amount of N_2O released was low, representing approximately 0.3% in LP treatment and 0.5% in LPC treatment of the total nitrogen output from the system. This release must be constantly checked. This gas is a potential promoter of the greenhouse effect. It is formed and emitted in a few cases during nitrification or most often in the process of incomplete denitrification (Klotz and Stein, 2008; Bortoli, *et al*., 2012). Several factors besides the composition of cultivated species act in the formation of these gases and their exchange with the atmosphere through the process of incomplete denitrification (Boyd, 2020). Therefore, to reduce greenhouse gas release rates, its important study the community of bacteria and other microorganisms that occur in the culture ponds.

Unaccounted for nitrogen is quite variable in budget studies. Some reported less than 20% (Martin *et al*., 1998; Sahu *et al*., 2013; Van Khoi and Fotedar, 2010; Sahu *et al*., 2015; David *et al*., 2017), while others noted values of ~11 to 66% (Boyd 1985; Paez-Osuna *et al*., 1999; Flickinger *et al*., 2019). In this work, some variables were not measured due to the complexity of the analyzes and/or difficulty in collection, such as loss of gas and entry of other compounds. This includes the volatilization of NH_3 (Gross *et al*., 1999), the leaf fall, flowers and dust in the ponds, the development of some small animals and plants within the ponds, the migration of animals that may enter the system, deposit, or consume nutrients and leave the system, predation of fish and prawn by terrestrial animals, and others (David *et al*., 2017a; Flickinger *et al*., 2019). Furthermore, unexplained nutrients can also result from inaccuracies in the techniques or equipment used to measure the content of each compartment, human errors during data collection and the great variability observed within and between ponds. Thus, new collection and analysis techniques must be adapted and tested in future experiments.

5. CONCLUSION

In conclusion, the mapping of nitrogen in each compartment of the two treatment systems contributed to the understanding of the cycle of this nutrient within the aquaculture ponds. The data showed that the main input of nutrients occurs through the offered diet, as it was demonstrated in previous studies. Therefore, feeding management may be the major driver to direct the process of nutrient use in aquaculture ponds. The two integrated cultures in this study were effective in converting nitrogen into harvested biomass, with values of ~40-42.8%. A large amount was accumulated at the bottom of the ponds. Oxygenated sediments benefited water conditions by oxidizing and detoxifying anaerobic metabolites before diffusing into the water column. The long period of water retention in the ponds allowed sedimentation, decomposition, and denitrification processes in the presence or absence of oxygen. Thus, the nitrogen was rapidly transformed through the aquatic nitrogen cycle and released into the atmosphere as molecular nitrogen gas. Therefore, both systems demonstrated great potential for optimizing the use of resources.

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CAPÍTULO 4

CARBON BUDGET OF *Astyanax lacustris* FARMING IN INTEGRATED CULTURE WITH *Macrobrachium rosenbergii* AND WITH *Macrobrachium rosenbergii* AND *Prochilodus lineatus*

ABSTRACT

This study aimed to map the carbon content in integrated aquaculture systems. The experimental design was completely randomized, with two treatments and six replications. Two integrated cultures were developed, being yellow tail lambari (*Astyanax lacustris*) and giant river prawn (*Macrobrachium rosenbergii*) (LP) in comparison with yellow tail lambari, giant river prawn and curimbatá (*Prochilodus lineatus*) (LPC). Respectively, in the LP and LPC treatments, carbon input the system mainly through the inlet water of ~59.3 and 57.3%, followed by the diet ~32.1 and 22.9%, and absorbed CO_2 ~8.3 and 17.9%. Carbon output by seepage water was ~49.5 and 49.7%, accumulated in the sediment was ~33.1 and 35.6%, outlet water ~5.9 and 4.4%, harvested animals ~8.7 and 7.5% and released gases ~2,7 and ~2.8%. The data suggest that integrated aquaculture in earthen ponds absorb large amounts of carbon from the nutrient-rich water source and the atmosphere. The addition of prawns and curimbatás that feed on the benthic fauna, detritus and diet remains offered to the target species increased the incorporation of carbon sources present in the harvested biomass, improving the efficiency of the systems.

KEYWORDS: carbon budget, integrated aquaculture, *Astyanax lacustris*, *Macrobrachium rosembergii*, *Prochilodus lineatus*.

1. INTRODUCTION

Aquaculture worldwide is moving towards an ecological approach to production that aims to improve yield, natural resource efficiency and halt the degradation of the planet (Boyd *et al*., 2020). Therefore, the use of fossil fuels, water, land and use of wild fish per unit of production of agricultural products must be reduced (Boyd *et al*., 2020). Improving the efficiency of integrated systems requires detailed knowledge of nutrient cycling. Thus, carrying out the activity with a scientific basis can increase sustainability, triggering the use of natural resources in replacing allochthonous feed (Boyd and Zimmerman, 2010; Valenti *et al*., 2018).

Carbon is a fundamental nutrient, being present in the diet of all animals and can be converted into harvested biomass. Portions not incorporated by animals can accumulate at the bottom of ponds through unused feed, feces and other sedimentation processes (Boyd, 2020). The remainder is converted into carbon dioxide (CO_2), which improves primary production and allows the expansion of the aquatic food chain, which leads to an increase in phytoplankton turnover (Moriarty, 1997; Boyd *et al*., 2010). Dead phytoplankton and other organic materials return to the bottom sediments, leading to anaerobic decomposition, which can cause the release of harmful metabolites (Boyd, 2020; Flickinger *et al*., 2020a).

Thus, knowledge of the carbon budget is necessary to understand the accumulation of this nutrient in aquaculture systems and to understand how farmed species can convert this essential nutrient into harvested biomass (Liu *et al*., 2014; Marques *et al*., 2016; Flickinger *et al*., 2020a; David *et al*., 2021). Advances in science, technology and innovation have indicated solutions that are intertwined with the sustainable development goals established in the 2030 Agenda (UN General Assembly, 2015). Thus, the production of aquatic organisms addressing current and future challenges and the aquaculture industry can contribute to long-term growth (Boyd *et al*., 2020). The sustainable use of freshwater has become a concern with the expansion of aquaculture, as freshwater resources are limited due to increased demand for activities (FAO, 2020).

Integrated species culture is a management strategy that aims to increase yield per production area through the culture of multiple organisms from different trophic levels that have complementary ecosystem functions (Chopin *et al*., 2012). This form of culture makes it possible to feed only the target species while one or more secondary species use the nutrients, residues and by-products originated from feeding the principal species (Chopin *et al*., 2013; Marques *et al*., 2016). This strategy optimizes the use of space, water, and other natural resources, such as energy and carbon sources (Diana *et al*., 2013; Marques *et al*., 2016). Thus, integrated aquaculture systems are a strategy to improve environmental sustainability and carbon utilization.

The culture of yellow tail lambari (*Astyanax lacustris*), giant river prawn (*Macrobrachium rosenbergii*) and curimbatá (*Prochilodus lineatus*) in an integrated system shows promise in this context. Whereas previous studies showed promising results using fish and prawn. However, even with the optimization of resource use, the cultures showed a high amount of nutrients accumulated at the bottom of the ponds

(Flickinger *et al*., 2019, 2020a, 2020b; David *et al*., 2017a, 2017b, 2021). This species showed promising productivity results in the integrated culture of tambaquis (*Colossoma macropomum*) and Amazon river prawn (*Macrobrachium amazonicum*) (Franchini *et al*., 2020) and of lambaris, Amazon river prawn and curimbatás (Marques *et al*., 2021). Thus, this work aimed to carry out the carbon budget in aquaculture production systems, using two species of fish and a specie of prawn.

2. MATERIAL AND METHODS

2.1. Experimental design

An experiment of integrated cultures was carried out at the Crustacean Sector of the Aquaculture Center, São Paulo State University, Brazil (21º15’22’’S e 48º18’48’’O). The experiment was conduct from January 14 to March 16, 2020, which is in the warm and rainy season. Twelve earthen ponds with area of ~0.015 ha and depth of 1 m were used. The sediment accumulated in previous experiments was not removed. Drainage, drying and replacement of water lost through seepage was carried out according to the method described in Chapter 2. The experimental design was completely randomized, consisting of two treatments and six replications. The treatments were integrated culture of yellow tail lambari (Astyanax lacustris) and giant river prawn (Macrobrachium rosenbergii) (LP), and integrated culture of yellow tail lambari, giant river prawn and curimbatá (Prochilodus lineatus) (LPC). The stocking density of the animals was lambaris = 40 ind.m-2, prawn = 5 ind.m-2 and curimbatás = 3 ind.m-2. The experiment lasted 62 days. This was the time required for lambari to reach the size of 7 cm to be marketed as live bait. Prawn can be marketed as human consumption and curimbatá as live fish to restocking native species by hydropower companies. The main justification for introducing this last species is the use of nutrients accumulated on the pond bottoms.

2.2. Monitoring of water variables

Temperature, dissolved oxygen, conductivity, and pH were measured daily at 8:00 a.m. and 4:00 p.m. with a YSI 556 Professional Plus multiparameter probe. Data are shown in Chapter 2. All values were suitable for the three farmed species.

2.3. Diet

The target species of the production was the yellow tail lambari, which was fed twice a day with 10% of its biomass. The diet provided was commercial feed for omnivorous fish (Guabi-Aqua Onívoros QS, Guabi Tech Juvenil – 36% crude protein, 2.3 mm granulometry). This diet is currently offered by Brazilian lambari producers because there is no commercial feed formulated for the species. Curimbatá and giant river prawn benefited from the nutrients, by-products and residues of the diet supplied to lambari.

2.4. Stocking and harvest

Stocking, harvest, and biometric procedures were described in Chapter 2. The ponds were populated with lambari fingerlings aged 15 days, prawn post-larvae aged 20 days and curimbatás aged 1 year. At the end of the experiment, all the ponds were dried and harvested. Data on animal size at stocking, yield, survival, and final mean weight of each species at harvest and feed conversion rate are shown in Table 1. These data were discussed in detail in Chapter 2.

Table 1. Mass, length, survival, yield, and Feed Conversion Ratio (FCR) obtained in each treatment. LP = integrated culture of lambari and prawn, LPC = integrated culture of lambari, prawn and curimbatá. *P* = probability to obtain the value of the *t*-statistic if the means are not different. Initial size: *A. lacustris* = 1.5 ± 0.2 cm; 0.04 ± 0.02 g; *M. rosenbergii* = 1.76 ± 0.44 cm; 0.05 ± 0.03 g; *P. lineatus* = 13.4 ± 1.3 cm; 27 ± 9.9 g.

	LP*	**LPC***	***P***
Total yield (t.ha^{-1})	1.9 ± 0.5	2.5 ± 0.3	0.092
Total diet supplied (kg/ha)	2.5 ± 0.8	2.4 ± 0.3	0.839
FCR	1.3 ± 0.5	1.5 ± 0.3	0.457
A. lacustris			
Mean final mass (g)	5.6 ± 1.9	6.2 ± 2.2	0.761
Mean final length (cm)	6.5 ± 0.9	6.8 ± 0.9	0.763
Survival (%)	70	62	-
Yield (t.ha^{-1})	1.5 ± 0.5	1.5 ± 0.4	0.861
M. rosenbergii			
Mean final mass (g)	8.9 ± 0.9 ^{a}	5.9 ± 1.2 ^{b}	0.007
Mean final length (cm)	9.8 ± 0.5 ^{a}	8.5 ± 0.6 ^{b}	0.008
Survival (%)	89	89	-
Yield (t.ha^{-1})	0.4 ± 0.03 ^{a}	0.3 ± 0.05 ^{b}	0.003
P. lineatus			
Mean final mass (g)	-	33.9 ± 4	-
Mean final length (cm)	-	13.6 ± 1.3	-
Survival (%)	-	75	-
Yield (t.ha^{-1})	-	0.8 ± 0.2	-

* Values followed by the same letter in a line did not differ statistically. Data are presented as mean ± standard deviation of the mean.

2.5. Sample collection for analysis of carbon

Sample collections were performed fortnightly over 60 days. Samples were obtained from the various ecological compartments of each pond as described below.

2.5.1. Water

Samples of supplied water and the water drained during harvests were collected in all ponds in both treatments. The seepage loss was measured according to the method described in Chapter 2. The water samples were analyzed in a TOC-C - Elementar analyzer to obtain the total carbon.

2.5.2. Gases

To determine the absorption and emission of CO_2 and CH_4 gases by diffusion and boiling (bubbles), five samples in each pond were obtained in the 62 days of the experiment (culture cycle). Samples were collected and stored according to the method described in Chapter 3. All samples collected by transfer tubes were analyzed in the GC-2014 Permanent Gas Analyzer - Shimadzu. Data are obtained in ppm and converted to kg C ha^{-1}. The results refer to the sum of the diffusive and boiling gases occurred during the culture.

2.5.3. Animals, sediment, and diet

Animals and sediment samples of each species inside each pond were collected in the stocking and harvesting according to the method described in Chapter 2. For determination of carbon content, the CHNS Elementary Analyzer - Vario MACRO Cube were used. The operation of this equipment is described in Chapter 3.

2.6. Carbon budget calculation

Carbon contents in all the inputs compartments were summed. Then, the total carbon outputs (TO) were subtracted from the total input (TI). The result corresponds to the unaccounted portion (UP). The equations used were:

a) $TI = IW + AG + SF + SP + D$

b) $TO = WO + SW + EG + HF + HP + S$

c) $UP = TO - TI$

in which, IW (inlet water), AG (absorbed gases), SF (stocked fish), SP (stocked prawn) and D (diet) refer to the content of carbon at the input compartments; and WO (water outlet), SW (seepage water), EG (emitted gases), HF (harvested fish), HP (harvested prawn) and S (sediment) refer to the carbon content at the outlet compartments.

2.7. Statistical analysis

All variables obtained were subjected to normality and homoscedasticity analyses through the Shapiro-Wilk and Bartlett tests, respectively. Data did not deviate from normality and homecedascity, and thus, they were subjected to statistical analysis using the *t*-test to verify the differences in the means between both treatments. Statistical analyses were carried out in R software (version 4.0.3), and the level of significance considered was $\alpha = 0.05$.

3. RESULTS

The input compartments did not show statistical differences between treatments (Table 2). Inlet water was the main carbon input in both treatments, accounting for approximately 59.3 and 57.3%, followed by diet 32.1 and 22.9% and absorbed CO_2 from atmosphere 8.3 and 17.9%, in the LP and LPC treatments, respectively (Figs. 1-2). Carbon inputs from other compartments ranged from 0.3% in the LP treatment to 2% in the LPC treatment. Respectively, in the LP and LPC treatments, the largest amount of carbon leave the system through the seepage water representing approximately 49.5 and 49.7%, the sediment compartment accumulated most of the carbon in the ponds ranging approximately from 33.1 and 35.6%, water outlet represented approximately 5.9 and 4.4% (Figs. 1-2), the harvested fish and prawn represented approximately 8.7 and 7.5% and gases released by bubbles and diffusion represented approximately 2.7 and 2.8%. Most of the gases were exchanged with the atmosphere by the diffusion process. Carbon emission by bubbles was low in all treatments (Table 2). Only the accumulated sediment and total output differed significantly between treatments ($P < 0.05$). The LPC treatment accumulated more carbon at the bottom of the ponds (Table 2).

In both treatments, part of the inlet carbon was incorporated into the harvested biomass of fish and prawns (Table 3). The assimilation of carbon in the biomass of the cultivated species represented, in the LP and LPC treatments, respectively, 28.1 and 26.6% of the diet offered in the culture and 9 and 6.1% of the total carbon input (Table

3). The budget showed negative values in both treatments, that is, the total carbon measured in the input compartments was lower than in the output compartments (Table 2). C:N ratio in the sediment was approximately 11 in LP and 13 in LPC treatments (Chapter 3). Unaccounted carbon in the input was 121.3 ± 403.1 kg/ha, that is, 3.7% in the LP treatment and 257.9 ± 1278.9 kg/ha, that is, 5.6% in the LPC treatment.

Table 2. Carbon content in each ecological compartment for the two farming systems. LP = integrated culture of lambari and prawn. LPC = integrated culture of lambari, prawn and curimbatá. *P* = probability to obtain the value of the *t*-statistic if the means are not different.

Ecological compartments (kg C ha^{-1})	**Treatments***		***P***
	LP	**LPC**	
Input			
Diet	1014.5 ± 339.8	1016.3 ± 126.4	0.992
Inlet water	1874.9 ± 531.9	2538.2 ± 1109.4	0.246
CO_2 absorbed	260.9 ± 249.5	792 ± 1307.7	0.386
CH_4 absorbed	8.3 ± 12.1	4.7 ± 7.4	0.590
Stocked lambari	1.9 ± 0.4	1.9 ± 0.2	0.874
Stocked prawn	0.3 ± 0.05	0.3 ± 0.03	0.874
Stocked curimbatá	-	80.2 ± 8.1	-
Total	3160.8 ± 617.4	4433.6 ± 1343.2	0.153
Output			
Water outlet	194.3 ± 12.2	201.5 ± 19.6	0.216
seepage water	1624.6 ± 535.5	2292 ± 1082.6	0.237
CO_2 released (bubbles)	16.5 ± 21.7	56.1 ± 90.1	0.244
CH_4 released (bubbles)	15.2 ± 11.5	17.4 ± 25.5	0.824
CO_2 released (diffusion)	48.4 ± 118.7	37 ± 90.6	0.870
CH_4 released (diffusion)	9.4 ± 11.8	17.3 ± 17.8	0.390
Harvested lambari	251.3 ± 119.1	250.6 ± 97.2	0.994
Harvested prawn	35.6 ± 15.5	21.5 ± 5.3	0.107
Harvested curimbatá	-	75.4 ± 17.5	-
Sediment	1086.5 ± 195.5 ^{a}	1642.1 ± 319.5 ^{b}	0.038
Total	3281.9 ± 530.9 ^{a}	4611 ± 1070.6 ^{b}	0.044
Unaccounted			
Input – Output	- 121.3 ± 403.1	- 257.9 ± 1278.9	0.799

* Values followed by the same letter in a line did not differ statistically. Data are presented as mean ± standard deviation of the mean.

Table 3. Carbon incorporated by animals based on the overall diet supplied and total carbon input throughout the culture cycle. LP = integrated culture of lambari and prawn. LPC = integrated culture of lambari, prawn and curimbatá. C = carbon. *P* = probability to obtain the value of the *t*-statistic if the means are not different.

Carbon incorporated by animals (%)	**Treatments**	
	LP	**LPC**
Diet-C		
Lambari	24.6	24.5
Prawn	3.5	2.1
Curimbatá	-	0
Total	28.1	26.6
Total input of C		
Lambari	7.9	5.6
Prawn	1.1	0.5
Curimbatá	-	0
Total	9	6.1

Figure 1. Carbon budget in integrated culture of *Astyanax lacustris* and *Macrobrachium rosenbergii* (LP). Values are shown in percentages based on the total input and output to pond.

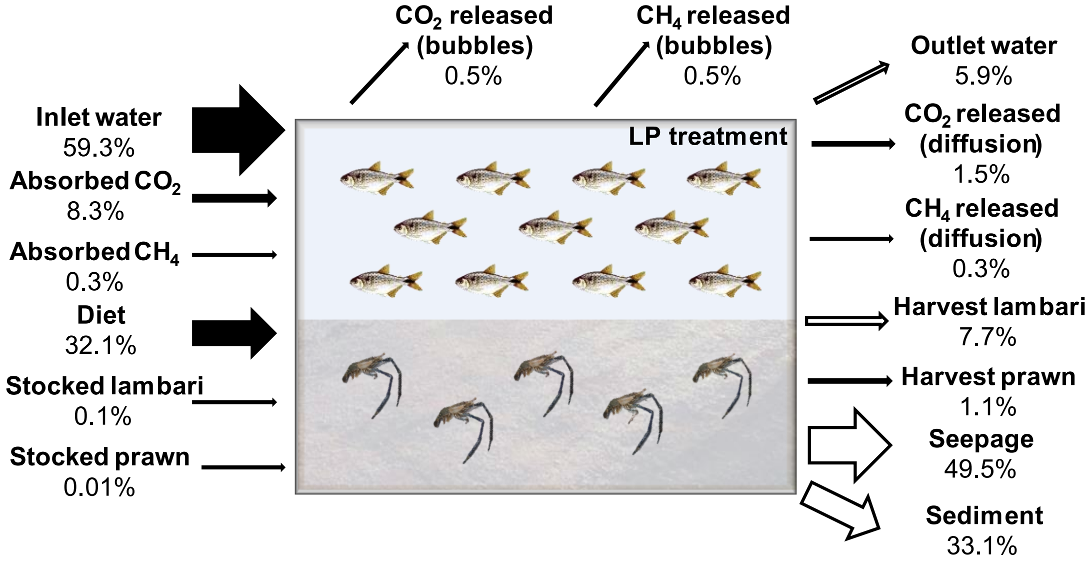
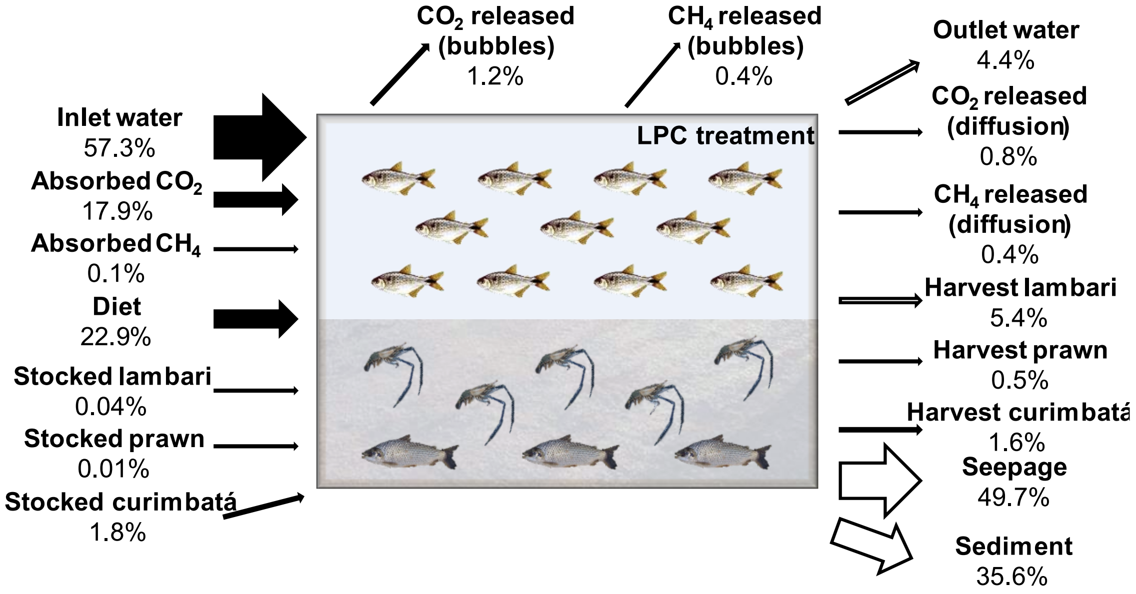


Figure 2. Carbon budget in integrated culture of *Astyanax lacustris*, *Macrobrachium rosenbergii* and *Prochilodus lineatus*. Values are shown in percentages based on the total input and output to pond.



4. DISCUSSION

The high carbon variability observed in the pond compartments decreased the power of the *t*-test (Sokal and Rohlf, 1995), and impairing the detection of differences between treatments. However, the carbon present in the incoming water was 9.7 times greater than that of the outgoing water in the LP treatment and 12.6 times in the LPC treatment, indicating a great assimilation of this nutrient by the earthen ponds. In addition, the absorption of atmospheric gases was 3 times greater than the emissions in the LP treatment and 6.2 times greater in the LPC treatment. Therefore, the two cultures of this work can be considered as an important provider of ecosystem services to mitigate the eutrophication of receiving water bodies and to absorb atmospheric gases. These results corrobrated those presented by Flickinger *et al*. (2020a), studying the integrated culture of *Colossoma macropomum* and *M. amazonicum*.

In the present study, water input represented the highest proportion of carbon input in both treatments (~59.3%; ~1874.9 ± 531.9 kg C ha^{-1} in LP and ~57.3%; ~2538.2 ± 1109.4 kg C ha^{-1} in LPC). Inlet water contributions reported at the same facility were higher than Amazon river prawn and tambaqui integrated culture (~18%) (Flickinger *et at*., 2020a) and Amazon river prawn and tilapia in integrated culture (~29-39%) (David, 2021). Conversely, other fish polyculture and *M. rosenbergii* monoculture studies have reported that inlet water contributed approximately 1% or less of all organic carbon inputs (Brown *et al*., 2012; Sahu *et al*., 2013a; Adhikari *et al*., 2014). The carbon contribution observed in the present study is due to the use of nutrient-rich water from a reservoir that receives effluents from previous cultures (Flickinger *et al*., 2019, 2020a, b; Dantas *et al*., 2020; Franchini *et al*., 2020; David *et al*., 2017a, b, 2021) combined with the large volume used to replace losses due to seepage and evaporation. The reuse of nutrient-rich water has been suggested as an alternative and may be a source for target organisms (Kimpara *et al*., 2011). The long period of water retention in the ponds allows carbon sedimentation and cycling processes (Boyd, 2020). Thus, both studied systems were efficient in removing carbon from the water.

The diet represented the second largest input into the system (~32.1%; 1014.5 ± 339.8 kg C ha^{-1} in LP) and (~22.9%; 1016.3 ± 126.4 kg C ha^{-1} in LPC). Diet contributions reported in the same facility were similar with the Amazon river prawn and tambaqui in integrated culture (~1799±325 kg C ha^{-1}), representing ~23 – 47% of input (Flickinger *et at*., 2020a) and less than the integrated culture of Amazon river prawn and tilapia (~58–63%) (David *et al*., 2021). The results were much lower when compared to previous studies carried out in other facilities that showed dietary carbon responsible for ~80 to 94% in *M. rosenbergii* and Pacific white prawn (*Litopenaeus vannamei*), respectively (Adhikari *et al*., 2014; Sahu *et al*., 2013a; Sahu *et al*., 2013b) and ~85 to 92% in integrated fish farming systems (Gal *et al*., 2013). The low proportion of carbon in the diet shown here, when compared to previous studies, is due to the high carbon content of the water source and the inclusion of carbon dioxide in the inputs. Allochthonous feeding can cause a reduction in dissolved oxygen levels, since the amount of diet provided is positively correlated with total carbon concentrations in the water column, indicating a high level of organic matter, which is associated with microbial decomposition (Avnimelech and Ritvo, 2003; Flickinger *et al*., 2020a). Therefore, the high amount of diet can affect the sedimentation process and harm the growth of species in aquaculture systems. Integrated culture is an important alternative to solve this problem because it optimizes the use of space and resources, reducing the amount of diet offered.

In the present study, gases had the third highest contribution to total carbon inputs (~8.3%; 269.2 ± 261.6 kg C ha^{-1} in LP) and (~18%; 796.7 ± 1315.1 kg C ha^{-1} in LPC). The values of CO_2 and CH_4 absorption are much higher than gas emissions. The low emission of gases and the high carbon content in the water suggest that photosynthetic organisms immobilized greenhouse gases. According to reported in the nitrogen budget study (Chapter 3), understanding the proportion of carbon to nitrogen (C:N ratio) in organic matter is a key factor in understanding the cycle of these nutrients in ponds and explaining gas emissions. Previous studies have reported that to function shift of the bacterial community in the sediment should in prawn and fish farming systems with C:N ratio at 12 (Roy *et al*., 2010; Rohmana *et al*., 2015; Perez-Fuentes *et al*., 2016; Zheng *et al*., 2018). In the present study, the C:N ratio in the sediment was 11 in the LP treatment and 13 in the LPC treatment. Consequently, possible changes occurring in the sediment's bacterial community can influence the diversity of the water column, directly influencing the absorption of gases by diffusion. These results are consistent with those observed by Flickinger *et al*. (2020a). The emission or absorption of greenhouse gases to/from the atmosphere are important factor for assessing sustainability in aquaculture (Boyd *et al*., 2010; Clark and Tilman, 2017). These gases were absorbed throughout the culture, indicating that the integrated systems studied can produce biomass and at the same time absorb greenhouse gases. These results support the hypothesis that earthen ponds used in aquaculture have a high capacity to absorb atmospheric gases and accumulate carbon as solid organic matter in bottom sediments with few adverse impacts on water quality (Boyd *et al*., 2010; Flickinger *et al*., 2020a).

The largest amount of carbon output was represented by seepage water, with approximately 50% in both treatments. This indicates that the system retains carbon, which is an environmental service (Valenti *et al*., 2018). The long period of water retention inside the earthen ponds allows sedimentation and decomposition processes (Boyd, 2020). Furthermore, the medium through which water must infiltrate to reach the permanently saturated groundwater zone is highly effective at filtering particles such as bacteria and adsorbing chemicals (Boyd, 2020). The second largest carbon output was through accumulation in the sediment. In this compartment, the integrated culture with the inclusion of curimbatá significantly accumulated more carbon. This result may be due to the increase in the bioturbation produced by the curimbatá, which expose more buried sources of organic carbon to aerobic decomposition and release of nutrients into the water column, increasing photosynthesis and carbon fixation, that sediment in the bottom (Green, 1992; Joyni *et al*., 2011; Flickinger *et al*., 2020a).

In previous studies in the same facilities, in integrated culture of tilapia and prawn, most of the carbon accumulated in the bottom sediments (~42 to 70%). Studies in other facilities have represented the same trend of large accumulation of organic carbon in the sediment, in monocultures of *M. rosenbergii* and *P. monodon*, representing ~64% of all carbon outputs (Sahu *et al*., 2013a, b; Adhikari *et al*., 2014). High concentrations of organic matter in pond sediment can lead to unsuitable conditions for aerobic decomposition and, consequently, reduced oxygen levels at the sediment-water interface (Boyd, 2020). The anaerobic decomposition of organic compounds runs the risk of accumulating harmful metabolites such as H_2S, NH_3 and CH_4 (Kassila, 2003), whose toxicity is more pronounced for prawn, since they live at the bottom of the ponds (David *et al*., 2021). In the present study, the results indicated a reduction in the carbon accumulated in the bottom. Therefore, it improves the use of nutrients in the sediment, reducing problems related to anaerobic metabolism.

In the LP treatment, ~9% of the carbon from all inputs were converted to biomass, while in the LPC treatment 6.1% of the carbon were recovered. The carbon conversion of the total input of *M. rosenbergii* in fed monocultures was ∼15-16% for (Sahu *et al*., 2013a; Adhikari *et al*., 2014). In the same facilities, similar data were obtained for Flickinger *et al*. (2020a), who reported values of 4% of the carbon of all carbon inputs into the system. David *et al*. (2021) reported higher values for tilapia and prawn in integrated culture, which recovered ~13 to 14% of the total carbon input. The low retention of organic carbon in the farmed species in the present study may be due to the high loss of carbon by seepage, which stems from the high age of the ponds. In addition, integrated cultures have species that feed on bottom fauna, fish feces and diet wastes making the carbon retained by prawn and curimbatá represent a gain of 100%.

The unaccounted carbon at the input was 121.3 ± 403.1 kg/ha, that is, 3.7% in the LP treatment and 257.9 ± 1278.9 kg/ha, that is, 5.6% in the LPC treatment. These values are lower than it was observed in tambaqui and prawn integrated culture, which represented ∼23 – 49% of inputs (Flickinger *et al*., 2020a). In integrated tilapia and prawn cultures with added substrates the unidentified portion of carbon ranged from ~1 to 27% of all outputs (David *et al*., 2021). Other carbon mass budget analyzes recorded ~17 to 19% of all carbon (Adhikari *et al*., 2014; Sahu *et al*., 2013a, b). Unaccounted carbon may be related to carbon uptake by small fish, insect larvae and other aquatic organisms unaccounted for, waterfowl consuming fish and prawn, and possible errors during analytical procedures.

5. CONCLUSION

The present work helped to understand the carbon budget in earthen ponds. Water represented the main nutrient input into the ponds due to the use of nutrient-rich water from a reservoir that receives effluents from previous cultures. The system showed great potential for retaining the carbon present in the water, which allows carbon sedimentation and cycling processes. Thus, combining the lambari and giant river prawn or these two species plus curimbatá were efficient in removing carbon from water despite the high accumulation of organic carbon in the sediment. The two treatments analyzed showed low emission and high absorption of greenhouse gases, indicating an increase with the addition of curimbatá. The addition of prawns and curimbatás that feed on the benthic fauna and the remains of the diet offered to the lambari increased the incorporation of carbon sources presented in the harvested biomass, improving the efficiency of the systems. Therefore, the addition of species from complementary ecological niches optimized the use of space, water resources and favored the absorption of greenhouse gases. Future research should be carried out to optimize the use of carbon present in the sediment and in the water column, including the verification of the bioturbation of the cultivated species in the re-availability of the nutrients present in the bottom.

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CAPÍTULO 6

CONCLUSÕES GERAIS

O balanço dos nutrientes nos compartimentos ecológicos estudados permitiu entender como esse nutriente se distribui nos cultivos integrados do lambari-do-rabo-amarelo e camarão-da-amazônia e destes mais o curimbatá. Os dados mostraram que os nutrientes entram no sistema de cultivo principalmente por meio da dieta (fósforo e nitrogênio) e da água de abastecimento (carbono) e os cultivos integrados apresentam grande capacidade de retenção desses nutrientes no sistema. A adição dos curimbatás que se alimentam da fauna bentônica e restos da dieta oferecida às espécies-alvo não aumentou a incorporação de nutrientes na biomassa colhida porque esses animais cresceram muito pouco dentro do sistema de cultivo. Portanto, não basta adicionar espécies para aumentar a eficiência do sistema. É necessário promover uma proporção adequada entre as espécies cultivadas

Os sistemas estudados demonstraram capacidade de absorção de gases do efeito estufa indicando maior absorção com a adição do curimbatá. As entradas de água foram significativas para todos os nutrientes e representou o maior aporte de carbono, superando inclusive a dieta fornecida para os lambaris. As duas combinações de espécies mostraram capacidade de retenção de nutrientes provenientes da água, que favoreceu processos de sedimentação, decomposição e ciclagem de nutrientes. A água drenada durante a despesca pode ser reutilizada para irrigar culturas terrestres e o sedimento do fundo pode ser usado para o cultivo de hortaliças ou outros vegetais.

Pesquisas futuras devem ser realizadas para entender o processo de bioturbação das espécies cultivadas na disponibilização dos nutrientes presentes no fundo. Além disso, estudos visando o aproveitamento de fósforo, nitrogênio e carbono presente no sedimento e na água liberada na despesca também são necessários.