

UNIVERSIDADE ESTADUAL PAULISTA – UNESP  
CENTRO DE AQUICULTURA DA UNESP

**BALANÇO DE FÓSFORO NO SISTEMA DE  
PRODUÇÃO DO LAMBARI-DO-RABO-  
AMARELO EM MONOCULTIVO E EM  
CULTIVOS INTEGRADOS COM CAMARÃO-  
DA-AMAZÔNIA E CURIMBATÁ**

**Julia Raquel do Carmo Ferreira**

Jaboticabal, São Paulo

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Dissertação apresentada ao  
Programa de Pós-graduação em  
Aquicultura do Centro de  
Aquicultura da UNESP - CAUNESP,  
como parte dos requisitos para a  
obtenção do título de Mestre em  
Aquicultura.

Jaboticabal, São Paulo  
2021

F383b Ferreira, Julia Raquel do Carmo  
Balanço de fósforo no sistema de produção do lambari-do-rabo-amarelo em monocultivo e em cultivos integrados com camarão-da-amazônia e curimatã / Julia Raquel do Carmo Ferreira. –  
Jaboticabal, 2021  
41 p. : il. ; 29 cm

Dissertação (mestrado) - Universidade Estadual Paulista, Centro de Aquicultura, 2021  
Orientador: Wagner Cotroni Valenti  
Banca examinadora: Dallas Lee Flickinger, Felipe Pereira de Almeida Cohen  
Bibliografia

1. Cultivo integrado. 2. Balanço de fósforo. 3. Aquicultura sustentável. 4. Lambari-do-rabo-amarelo. 5. Camarão-da-amazônia. 6. Curimatã. I. Título. II. Jaboticabal-Centro de Aquicultura.

CDU 639.3.05

### **APOIO INSTITUCIONAL E FINANCEIRO**

Agradeço ao Centro de Aquicultura da UNESP (CAUNESP) pelo apoio institucional ao longo do desenvolvimento dessa dissertação. E aos financiadores da Rede de Pesquisa de Sustentabilidade, na qual esse projeto está inserido (Edital MCT / CNPq / MEC / CAPES / CT AGRO / CT HIDRO / FAPS / EMBRAPA No 22/2010 – REPENSA; CNPq Processo 562820/2010-8; 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). À Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP, pelo auxílio financeiro para desenvolvimento desta pesquisa - 10/52210-3. O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

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## RESUMO

A sustentabilidade na aquicultura pode ser aumentada por meio de cultivos integrados e multitróficos. O fósforo é um fator limitante na produção de organismos aquáticos. Neste trabalho, foi testada a hipótese de que o cultivo integrado com espécies de diferentes nichos tróficos aumenta a assimilação de fósforo no sistema. Um experimento de 60 dias foi realizado em 12 viveiros de fundo natural, com três tratamentos e quatro repetições, todos em sistema fechado, sendo eles o sistema de monocultivo de lambari-do-rabo-amarelo (*Astyanax lacustris*) e os cultivos integrados das espécies *A. lacustris* com camarão-da-amazônia (*Macrobrachium amazonicum*) e *A. lacustris* com *M. amazonicum* e curimbatá (*Prochilodus lineatus*). O lambari exerceu a função de espécie principal, sendo diretamente alimentado com ração comercial (32% de proteína bruta) duas vezes ao dia. O camarão-da-amazônia e o curimbatá foram as espécies secundárias, se alimentando dos resíduos produzidos no sistema, como ração e fezes, e da biota aquática. As entradas e saídas de fósforo no sistema foram quantificadas por meio de amostras de água de entrada, saída e da chuva, dos animais, do sedimento e da ração fornecida para determinar a massa de fósforo acumulada em cada compartimento do sistema. Nenhum compartimento de entrada apresentou diferença estatística entre os tratamentos. A dieta foi responsável pela maior entrada de fósforo no sistema (60 – 64%), seguido pela água de abastecimento (18 – 29%) que é hipereutrófica. Na saída, o sedimento representou 69 – 81% do fósforo, mostrando que há um acúmulo do que não é assimilado pelo sistema nesse compartimento. A assimilação de fósforo apresentou valores de 51,3% no monocultivo, 56,35% no cultivo integrado de *M. amazonicum* e *A. lacustris*. O maior valor encontrado foi de 78,9% no cultivo integrado com as três espécies. Esse estudo confirma a hipótese inicial e mostra que existe uma otimização do uso dos recursos e aproveitamento de nutrientes quando são usados sistemas multitróficos com espécies com características ecológicas complementares.

**Palavras-chave:** cultivo integrado, balanço de fósforo, aquicultura sustentável, lambari-do-rabo-amarelo, camarão-da-amazônia, curimbatá.

## ABSTRACT

Aquaculture sustainability can be increased through integrated and multitrophic aquaculture. Phosphorus is a limiting factor in the production of aquatic organisms. In this work, we tested the hypothesis that integrated multitrophic aquaculture systems with species from different trophic niches increases the assimilation of phosphorus in the system. A 60-day experiment was carried out in 12 earthen ponds, with three treatments and four replications in closed system. They were the monoculture of yellow tail lambari (*Astyanax lacustris*), the integrated culture with lambari and Amazonian river prawn (*Macrobrachium amazonicum*) and the integrated culture with lambari, prawn and curimbata (*Prochilodus lineatus*). Lambari were fed commercial feed (32% crude protein) twice a day. The other species benefited from the wastes produced in the system and nutrients from inlet water. Phosphorus inputs and outputs in the system were quantified using samples of the inlet, outlet, and rain water, animals, sediment, and feed. The mass of phosphorus accumulated in each compartment of the system was determined. The entrance compartments showed no statistical difference among treatments. The diet accounted for the majority phosphorus entrance in the system (60 - 64%), followed by inlet water (18 - 29%). The sediment showed statistical difference and accumulated 69 - 81% of the phosphorus, showing that there is an accumulation of what is not assimilated by the system in this compartment. Phosphorus assimilation showed high values in the farmed species, representing 51.3% in monoculture, 56.35% in integrated cultivation of *M. amazonicum* and *A. lacustris* and the highest value found was 78.9% in integrated cultivation with the three species. This study confirmed the hypothesis that there is an optimization of the use of resources and nutrients when in multitrophic systems.

**Keywords:** Integrated multitrophic aquaculture, phosphorus budget, sustainability, yellow tail lambari, Amazon River prawn, curimbata.



## CAPÍTULO 1

### 1. INTRODUÇÃO GERAL

A aquicultura é um segmento que cresce a cada ano no Brasil. Em 2020 atingiu uma produção de 802,9 mil toneladas, crescendo 5,93% em relação ao ano anterior, segundo a associação nacional de produtores de peixes, Peixe BR (Peixe BR, 2020). Os dados oficiais fornecidos pelo Instituto Brasileiro de Geografia e Estatística (IBGE, 2021) indicam produção de 612,5 mil toneladas no ano de 2019. O cultivo de diversas espécies de peixes, camarões, moluscos e outros organismos aquáticos vêm sendo explorado nos últimos anos devido ao seu potencial econômico, como o uso na alimentação humana, iscas vivas, ornamentação e também o uso de seus subprodutos.

Para que seja garantido que as gerações humanas presentes e futuras tenham suas necessidades atendidas, é necessária uma boa gestão dos recursos já existentes, tanto no âmbito financeiro, natural e social, quanto no tecnológico e institucional. Isso é o que define a sustentabilidade (Valenti et al., 2018). A aplicação de práticas sustentáveis não necessariamente torna um cultivo sustentável (Belton et al., 2009), no entanto a adoção dessas práticas se torna cada vez mais importante para minimizar os efeitos negativos de uma produção. Diversos tipos de análises são viáveis para mensurar a sustentabilidade da aquicultura, como a síntese emergética (Odum & Arding, 1991), pegada ecológica (Wackernagel & Rees, 1998) e o uso dos indicadores de sustentabilidade (Valenti et al., 2018). Marques et al. (2016) define o cultivo integrado como qualquer sistema de aquicultura que compartilha seus recursos com outros sistemas aquícolas ou com outras atividades econômicas. O sistema multitrófico coloca espécies de diferentes níveis tróficos com funções ecológicas complementares num mesmo cultivo, de modo que os alimentos e/ou os resíduos não consumidos pela espécie principal sejam reaproveitados pelas espécies secundárias em forma de fertilizante, alimento e energia (Chopin et al., 2013). Deste modo, o cultivo multitrófico integrado surge visando maximizar o aproveitamento dos nutrientes e energia disponíveis, e reduzir o impacto ambiental.

O cultivo integrado multitrófico de espécies como *Astyanax lacustris* (lambari-do-rabo-amarelo), *Macrobrachium amazonicum* (camarão-da-

amazônia) e *Prochilodus lineatus* (curimbatá) parece promissor devido às características das mesmas. O lambari é um animal pelágico com hábitos onívoros oportunistas também servindo de alimento para espécies carnívoras de maior porte (Garutti, 2003). O camarão-da-amazônia é uma espécie bentônica e detritívora, sendo usada com sucesso em cultivos integrados com peixes (Maciel e Valenti, 2009; David et al., 2017a, b; Flickinger et al., 2019). O curimbatá foi descrito por Fugui et al. (2001) como um animal detritívoro e herbívoro. Os principais componentes encontrados no seu estômago foram sedimento e detritos, evidenciando seu hábito iliófago. Isto sugere que o curimbatá é uma boa alternativa nesse cultivo integrado, podendo incorporar nutrientes que antes ficariam disponíveis na água e no sedimento. As três espécies juntas mostram um grande potencial de cultivo integrado, visto que todas são nativas, minimizando o risco de contaminação ambiental por patógenos ou de introdução de espécie exótica no meio ambiente por meio de escapes.

O fósforo é um recurso natural indispensável para a manutenção da vida. Esse nutriente está presente em diversos processos metabólicos e reações bioquímicas. No formato de fosfato inorgânico ele participa de funções essenciais como formação dos ossos, além de ser parte fundamental dos ácidos ribonucleicos, RNA e DNA (Corbridge, 2013a, b). É um fator limitante na produção de organismos aquáticos e terrestres, sendo um dos maiores responsáveis pelo processo de eutrofização, mas é essencial nesses sistemas (Flickinger et al., 2019), por isso é importante para avaliar a sustentabilidade, segundo o conjunto de indicadores proposto por Valenti et al. (2018). Praticamente ausente na atmosfera, sua maior fonte primária é o escoamento de bacias hidrográficas e a dissolução do fósforo das rochas e dos sedimentos. Quando não aproveitado por plantas e bactérias, ele é absorvido pelo solo como fosfato de ferro, alumínio e cálcio e adsorvido como hidróxidos e óxidos de ferro e alumínio. E é dessa forma que ele é encontrado na natureza, tendo alta ocorrência em concentrações baixas de fosfato de ferro e alumínio, mas em grandes concentrações de fosfato de cálcio em poucas localidades. A apatita, mais conhecida como “rocha de fosfato”, é formada por esse depósito de fosfato de cálcio. Essa rocha passa por diversos processos para que o fósforo seja disponibilizado de uma forma mais solúvel e assim utilizado na

indústria e no comércio, sendo componente importante na confecção de pesticidas e fertilizantes. O ciclo de fósforo dentro de um sistema ocorre quando o fósforo inorgânico dissolvido é absorvido e incorporado pelas plantas, que são consumidas pelos organismos herbívoros e onívoros ali presentes, entrando assim na cadeia alimentar. Quando acontece a morte dos mesmos, a atividade microbiana mineraliza o fósforo de suas carcaças e o que não é absorvido pelas plantas é adsorvido pelo sedimento (Boyd, 2015; Boyd, 2020; de Boer, 2018).

Este trabalho foi desenvolvido com outros dois que avaliaram a) o balanço energético do sistema de monocultivo e dos dois sistemas de cultivo integrado, e b) o balanço de nitrogênio do sistema de monocultivo e dos dois sistemas de cultivo integrado. Também foram desenvolvidos outros dois que avaliarão a) a sustentabilidade ambiental, social e econômica dos três sistemas, por meio de indicadores de sustentabilidade; b) a contribuição das fontes de alimento disponíveis para as espécies cultivadas, por meio do uso de isótopos estáveis. A intenção foi de agrupar a maior quantidade de informações desse cultivo, tendo um maior aproveitamento científico dos dados obtidos, otimizando os recursos investidos na pesquisa e as instalações do projeto. Portanto, nesse projeto foram realizadas análises atuais e muito importantes para a sustentabilidade ambiental, para subsidiar pesquisas futuras e políticas públicas.

## **2. OBJETIVO GERAL**

Avaliar o balanço de fósforo nos sistemas de monocultivo de *A. lacustris*, sistema integrado de *A. lacustris* e *M. amazonicum* e sistema integrado de *A. lacustris*, *M. amazonicum* e *P. lineatus* para testar a hipótese de que maior quantidade de fósforo se acumula no compartimento dos animais quando espécies com nichos tróficos complementares são cultivadas em conjunto.

## **3. OBJETIVOS ESPECÍFICOS**

- Quantificar o fósforo em todos os compartimentos que compõem os sistemas de monocultivo e cultivos integrados.
- Avaliar a assimilação e eficiência de fósforo pelas espécies alvo nos diferentes sistemas de cultivo.

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## CAPÍTULO 2

### PHOSPHORUS BUDGET OF YELLOW TAIL LAMBARI FARMED IN MONOCULTURE AND INTEGRATED MULTITROPHIC SYSTEMS WITH AMAZON RIVER PRAWN AND CURIMBATA

#### ABSTRACT

Aquaculture sustainability can be increased through integrated and multitrophic aquaculture. Phosphorus is a limiting factor in the production of aquatic organisms. In this work, we tested the hypothesis that integrated multitrophic aquaculture systems may increase the assimilation of phosphorus in the system. A 60-day experiment was carried out in 12 earthen ponds, with three treatments and four replications in a closed system. The treatments were the monoculture of yellow tail lambari (*Astyanax lacustris*), the integrated culture with lambari and Amazonian river prawn (*Macrobrachium amazonicum*) and the integrated culture with lambari, prawn and curimbatá (*Prochilodus lineatus*). Lambari were fed commercial feed (32% crude protein) twice a day. The other species benefited from the wastes produced in the system and nutrients from inlet water. Phosphorus inputs and outputs in the system were quantified using samples of water (inlet, outlet, and rain), animals, sediment, and feed. The mass of phosphorus accumulated in each compartment of the system was measured. The input compartments showed no statistical difference among treatments. The diet accounted for the majority phosphorus input in the system (60 - 64%), followed by inlet water (18 - 29%). The sediment showed statistical difference and accumulated 69 - 81% of the phosphorus, showing that there is an accumulation of what is not assimilated by the system in this compartment. The higher assimilation of phosphorus (78.9%) was found in the integrated culture with the three species, followed by the integrated cultivation of lambari and prawn (56.4%), and monoculture (51.3%). Prawn incorporated less phosphorus in presence of curimbatá. It suggests a competition by phosphorus, although prawn mass did not differ between treatments. This study supports the hypothesis that integrated multitrophic aquaculture can optimize of the use of resources and nutrients.

**Keywords:** IMTA, *Astyanax lacustris*, *Macrobrachium amazonicum*, *Prochilodus lineatus*, nutrient recycling, resources optimization, phosphorus use.

## 1. INTRODUCTION

The global population growth is expected to reach 9.7 billion people in 2050 (UN, 2019). One of the global challenges to be faced with this population increase is how to food everyone in. Besides that, a sustainable way to overcome this challenge is important to go through climate changes so prominent. Most agriculture activities are terrestrial and may cause many negative effects to environment, such as high land use and greenhouse gases emissions. Sustainable aquaculture comes as an economic and ecologic alternative to this problem (Béné et al., 2014; Boyd et al., 2020), considering that aquaculture produced 120.1 million tonnes in 2019 according to FAO (2021).

The choice of species is crucial to the development of a culture, in terms of resources use and productivity. To improve sustainability, one of the factors that may impact positively the production is to prefer natives species instead of exotic ones, because they can spread pathogens and microorganisms by scape, besides that, the biodiversity may be affected through competition and predation of native species (Valenti et al., 2021). Yellow tail lambari (*Astyanax lacustris*) is a pelagic fish species and has been produced to supply the market of live bait and seafood. It can be an important economic income alternative to small rural producers because of its easy management and short life cycle, reaching adult size between three and four months (Garutti, 2003; Silva et al., 2011a, b; Gonçalves et al., 2015; Fonseca et al., 2017). Lambari experienced the highest increase of aquatic organism culture from 2016 to 2019 in Brazil when compared to other aquatic species (IBGE, 2019). This species production is estimated at above 1,000 tonnes per year (Valenti et al., 2021). Amazon river prawn (*Macrobrachium amazonicum*) is a benthic animal commonly exploited by artisanal fisherman and it is sold as live bait and for human consumption (Moraes-Rioldades and Valenti, 2001). In 2019, brazilian production was 54.3 tonnes (IBGE, 2019 (dados de 2019, acessado em 2021)). The group of *Prochilodus* spp is the fifth most produced in Brazil among all native species.

These species can be released in hydrological basins impacted by dam and sold to be used as bait-fish when reaches ~15 cm (Valenti, 2021). Curimbatá (*Prochilodus lineatus*) has an iliophagous feeding habit, and shows demand in international markets. The curimbatá is the second most exported species from Brazil (Peixe BR, 2020). The association among these three native species can be economic feasible to producers who aim more profit and products variety, encouraging a better use of available resources and the development of a sustainable production, once each of them occupy distinct ecological niche and have complementary functions.

Aquaculture systems may generate economic circulation and improve the quality of the available food. However, aquaculture can also have negative environmental impacts that deserve some attention, as the wastage of food that may decrease the water quality of effluents (Sonesson et al., 2010). Sustainable aquaculture comes as an opportunity to reduce and mitigate negative effects of aquatic food production. In the past few years some socio-economic and environmental indicators were identified to measure the culture sustainability (Valenti et al., 2018). The implementation of an integrated multitrophic aquaculture (IMTA) systems in which only one species is directly fed and the others rely on leftover food, feces and autochthonous nutrients can be an opportunity to optimize the use of resources and increase its sustainability (Chopin et al., 2012). For a better management of these complex IMTA systems, in order to increase the efficiency of these production systems, it is fundamental to understand some essential nutrients functions that are important to the ponds, such as phosphorus, that can be pollutant when it is present in excess.

Phosphorus is an important nutrient to life management, it is present in several metabolic processes and biochemical reactions, being also part of coenzymes, phospholipids and nucleic acids, RNA and DNA (Corbridge, 2013a, b). Practically absent in atmosphere, its main source in water is the runoff from watershed soils and the dissolution of this nutrient present in sediment (Boyd, 2020). Phosphorus is mainly originated from phosphate rock, composed by a deposition of calcium phosphate (de Boer, 2018). After some processes of conversion into elemental phosphorus to industrial purpose, this element is introduced in aquaculture systems via fertilizers, feed and rainwater (Funge-



Smith and Briggs, 1998; de Boer, 2018). Phosphorus is a limiting nutrient in freshwater systems production (Flickinger et al., 2019). It is introduced intentionally as fertilizers which contains calcium or ammonium phosphate to stimulate phytoplankton growth in cultures that the organisms need natural feeding sources (Boyd, 1990; 2002; 2020). Phytoplankton rapidly removes phosphorus from the water and incorporates this nutrient on food chain by serving as food to fishes and prawns (Boyd, 2020). However, about 2/3 of phosphorus introduced by feed is accumulated at the bottom of the ponds (Masuda and Boyd, 1994; David et al., 2017; Flickinger et al., 2020). This nutrient, in the soil, usually becomes available if rain occurs when the pond is empty or by the harvest processes and animal habits that resuspend particles (Masuda and Boyd, 1994; Boyd, 2020). Phosphate is initially absorbed by soil, then fertilizer is added once more by producers to improve its availability in the ponds (Boyd, 2020). Phosphorus output occurs in sediment removal, water output, harvest and intentional withdrawal of water after culture (Funge-Smith and Briggs, 1998; Boyd, 2020). Because most phosphorus are in the sediment, adding benthic species that are omnivorous and detritivorous, as prawn and curimatá, in IMTA with lambari, can be a good opportunity to recovery phosphorus in pond bottom. Thus, this study aimed to evaluate the phosphorus budget in IMTA system with lambari, prawn and curimatá in comparison with lambari monoculture and IMTA system with lambari and prawn.

## **2. MATERIAL AND METHODS**

### **2.1. Pond preparation and management**

The experiment was carried out at the Crustacean Sector of the Aquaculture Center (CAUNESP), São Paulo State University, Brazil (21°15'22" S, 48°18'48" W). The experiment was conduct from February 4, 2019 to April 5, 2019. This is in the warm and rainy season. The study was conducted in 12 earthen ponds, each with an area of approximately 0.015 ha (150 m<sup>2</sup>) and a water depth of 1m. The ponds were drained, air-dried and then supplied with eutrophic water (0.14 mg P/L) (Brown and Simpson, 2001) from two reservoirs that receive effluents from other aquaculture systems. The ponds were kept in a closed system without water renewal, and the supply water was used only to replace losses to the evaporation and seepage. Each pond was provided with

an aerator (Bernauer, model B-500 Aquahobby monophasic – freshwater) to maintain adequate levels of dissolved oxygen. Aerators were turned on when DO was below 1.5 mg/L.

## **2.2. Experimental design**

The experimental design was completely randomized, consisting of three treatments and four replications. The treatments were monoculture of *Astyanax lacustris* (50 ind/m<sup>2</sup>) (LM), integrated culture of *A. lacustris* (50 ind/m<sup>2</sup>) and *Macrobrachium amazonicum* (25 ind/m<sup>2</sup>) (LP), and integrated culture of *A. lacustris* (50 ind/m<sup>2</sup>), *M. amazonicum* (25 ind/m<sup>2</sup>) and *Prochilodus lineatus* (13 ind/m<sup>2</sup>) (LPC).

## **2.3. Monitoring of water variables**

Some physical and chemical water variables of interest to aquaculture were monitored *in situ* (at 20 – 30 cm below the water surface) daily at 8:00 a.m. using a YSI 556 Professional Plus multiparameter probe. The variables analyzed were dissolved oxygen (DO, mg/L), temperature (°C), electrical conductivity (μS/cm<sup>2</sup>) and pH. Total phosphorus was determined biweekly by persulfate method (American Public Health Association, 2017, 4500-P B5) to liberate orthophosphates associated with organic material. Then, the orthophosphate was measured using the stannous chloride method (American Public Health Association, 2017, 4500-P D) and a mass spectrophotometer (UV-1000, Shimadzu) (Table 1).

**Table 1.** Water quality parameters (mean  $\pm$  SD) monitored during the grow-out of the yellow tail lambari (*Astyanax lacustris*) in monoculture system (LM) and fish-prawn IMTA systems with yellow tail lambari and Amazon river prawn (*Macrobrachium amazonicum*) (LP), and yellow tail lambari, Amazon river prawn and curimatá (*Prochilodus lineatus*) (LPC).

|                                                        | LM                                        | LP                                        | LPC                                       |
|--------------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------|
| <b>Temperature (°C)</b>                                | 27.6 $\pm$ 0.2<br>(27.4 – 27.8)           | 27.9 $\pm$ 0.2<br>(27.6 – 28.1)           | 27.9 $\pm$ 0.2<br>(27.6 – 28.0)           |
| <b>DO (mg/L)</b>                                       | 4.8 $\pm$ 0.3 <sup>a</sup><br>(4.5 – 5.2) | 6.0 $\pm$ 0.5 <sup>b</sup><br>(5.3 – 6.6) | 5.4 $\pm$ 0.2 <sup>a</sup><br>(5.0 – 5.5) |
| <b>pH</b>                                              | 8.0 $\pm$ 0.1<br>(7.9 – 8.0)              | 8.1 $\pm$ 0.4<br>(7.9 – 8.7)              | 8.1 $\pm$ 0.2<br>(8.0 – 8.3)              |
| <b>Conductivity (<math>\mu</math>S/cm<sup>2</sup>)</b> | 136 $\pm$ 1<br>(135 – 137)                | 134 $\pm$ 1<br>(133 – 136)                | 136 $\pm$ 1<br>(135 – 137)                |
| <b>Total nitrogen (mg/L)</b>                           |                                           |                                           |                                           |
| <b>Initial*</b>                                        | 0.41 $\pm$ 0.07<br>(0.34 – 0.50)          | 0.41 $\pm$ 0.07<br>(0.34 – 0.50)          | 0.41 $\pm$ 0.07<br>(0.34 – 0.50)          |
| <b>Final</b>                                           | 0.70 $\pm$ 0.21<br>(0.44 – 0.95)          | 0.57 $\pm$ 0.12<br>(0.40 – 0.68)          | 0.62 $\pm$ 0.12<br>(0.54 – 0.80)          |
| <b>Total phosphorus (mg/L)</b>                         |                                           |                                           |                                           |
| <b>Initial*</b>                                        | 0.14 $\pm$ 0.04<br>(0.11 – 0.20)          | 0.14 $\pm$ 0.04<br>(0.11 – 0.20)          | 0.14 $\pm$ 0.04<br>(0.11 – 0.20)          |
| <b>Final</b>                                           | 0.18 $\pm$ 0.07<br>(0.11 – 0.29)          | 0.20 $\pm$ 0.08<br>(0.13 – 0.28)          | 0.18 $\pm$ 0.03<br>(0.14 – 0.22)          |

Note: Minimum and maximum values of each measurement are shown in brackets. Initial = at the beginning of the experiment; Final = at the end of the experiment.

\* Measured in the inlet water used to fill all ponds.

## 2.4. Animal obtaining and diet

Lambari fingerlings were obtained from the commercial fish-farming of Sítio Sobradinho, Buritizal city, São Paulo state, Brazil (20°11'28" S, 47°42'30" W). Post larvae of prawn were obtained at the Crustacean Sector of the Aquaculture Center (CAUNESP), São Paulo State University, Brazil (21°15'22" S, 48°18'48" W). Curimatá fingerlings were obtained from the commercial fish-farming Projeto Peixes, Sales de Oliveira city, also in São Paulo state, Brazil

(20°46'19" S, 47°50'16" W). Feeding was based on lambaris' biomass only. It was used commercial diet with 32% of crude protein and granules of 2.5 mm (GuabiTech Aqua Onívoros QS) twice a day (Fonseca et al., 2017). The feeding was carried out for 53 days, not including the days of stocking and harvesting. For the first 31 days, the diet provided was 10% of the total biomass of the stocked lambari. After this period, a lambari biometrics was performed to adjust the feeding, which corresponded to 5% of the total lambari biomass. The prawns and curimbatá were supposed to rely on leftover food, autochthonous biota and waste nutrients produced in the system.

## **2.5. Zootechnical performance and harvest**

The initial, intermediate and final mass and length was obtained with a random sample of 100 individuals of each species from each pond. At the end of 60 days, all ponds were harvested and all animals were counted (Marques, 2021).

No statistical significant differences between treatments were obtained for the mean mass, length and survival of lambari and prawn, and for diet supplied during culture. Feed Conversion Ratio decreased progressively with the addition of prawn and curimbatá (Marques, 2021).

## **2.6. Phosphorus budget**

We analyzed the main ecological compartments of the inputs and outputs of phosphorus. The difference between the total phosphorus input (TPin) and the total phosphorus output (TPout) determined the unaccounted portion (UP). The equations used were:

$$\begin{aligned} \text{TPin} &= \text{IW} + \text{R} + \text{SL} + \text{SP} + \text{SC} + \text{D} \\ \text{TPout} &= \text{OW} + \text{HL} + \text{HP} + \text{HC} + \text{S} + \text{SW} \\ \text{UP} &= \text{TPin} - \text{TPout} \end{aligned}$$

In which inlet water (IW), rainwater (R), commercial diet (D), stocked lambari (SL), stocked prawn (SP), stocked curimbatá (SC) and commercial diet (D) refer to the phosphorus contents of the input compartments, and outlet water (OW), harvested lambari (HL), harvested prawn (HP), harvest curimbatá (HC), sediment (S) and seepage water (SW) refer to the phosphorus content of the output compartments. A negative UP means that unaccounted portion corresponds to input, while a positive UP means that unaccounted portion

corresponds to output. The assimilation was determined by total phosphorus content accumulated in each species of fish and prawn divided by diet phosphorus load (feed phosphorus conversion [FPC]). Efficiency of phosphorus use was determined by total phosphorus content accumulated in each species of fish and prawn divided by the sum of phosphorus in all input compartments (phosphorus use efficiency [PUE]).

The mass of phosphorus and the samples for analysis from the various ecological compartments of each pond were obtained as described below.

### **2.6.1. Water**

Samples of supplied water and the water drained during harvests were collected at the 12 ponds. Outlet water was collected on the day of harvest. Samples of inlet and pond column water was collected biweekly, stored in polyethylene bottles and kept in freezer (-18°C) until analyses. To estimate the seepage loss and thus measure the amount of replacement every two weeks, the pond inlet water was closed for a period of eight hours. A clapboard with a measuring tape was placed inside the pond. The height (m) was measured at 8:00 a.m. and 4:00 p.m. Based on the difference in water level and pond area, the volume lost by seepage was calculated. Rainwater samples were collected to determinate phosphorus concentration.

Evaporation and precipitation water data were extracted of a database from the Departure of Exact Sciences in an Agrometeorology area. Observations made on Agroclimatological Station on Jaboticabal Campus (21°14'05" S, 48°17'09" W, alt. 615,01m) are listed, typed in a pattern form, with consistence and quality control. Then, diary, mensal and annual means passed on to users are obtained. These values were discounted of seepage measurement to phosphorus quantification.

Input and output water phosphorus load was calculated by multiplying phosphorus concentration in water by total volume of input and output water of each pond. Seepage water phosphorus was calculated by multiplying phosphorus concentration in water by total volume of seepage water of each pond. Phosphorus concentrations were determined by persulfate method (APHA, 2017, 4005-P B5) and stannous chloride method (APHA, 2017, 4500-P D) using a mass spectrophotometer (ASC-5 and UV-1000, Shimadzu). Total

precipitation volume in the culture period was adjusted for each pond area and then multiplied by the mean phosphorus in rainwater.

#### **2.6.2. Animals and diet**

Samples of 5 g of each species per pond were collected in the stocking, and five individuals of each species per pond were collected in the harvesting. This was the quantity required to have a representative sample to perform the required analyses. The collected animals were slaughtered on ice and stored in properly identified plastic bags. 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. 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 phosphorus for all species were summed.

#### **2.6.3. Sediment**

Tripton collectors were used biweekly to collect sediment in the pond. 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 for 24h. Then, the sedimented material was removed from each PVC pipe, mixed and transferred to polyethylene bottles and kept in freezer (-18°C). The samples were transferred to Petri dish and dried in a forced air oven (Nova Ética – 700-7DE) at 60°C until reaching a constant mass. For determination of phosphorus content, the same methodology from animals and diet was used.

#### **2.6.4. Phosphorus incorporation by fishes and prawn**

The assimilation was determined by total phosphorus content accumulated in each species of fish and prawn divided by diet phosphorus load.

Efficiency of phosphorus use was determined by total phosphorus content accumulated in each species of fish and prawn divided by the sum of phosphorus in all input compartments.

## **2.7. Data analyses**

All variables obtained were subjected to normality and homoscedasticity analyses through the Shapiro-Wilk and Brown-Forsythe tests, respectively. When both conditions were satisfied, data were subjected to one-way ANOVA (*F*-test) (Sokal and Rohlf, 1995) to verify the differences in water-quality parameters, production, compartments of the phosphorus budget among the treatments and phosphorus incorporation by fishes and prawn. Assimilation and efficiency data for prawn were not normal and were subjected to a Kruskal-Wallis test. When significant differences were detected among treatments, means were compared with Fisher-LSD test. Curimatá data were subjected to a statistical description of its means and standard deviation, considering that this species was present only in one treatment. Linear regression was calculated to analyze the correlation among the number of species and phosphorus output and input by all compartments, phosphorus output and input sum, efficiency use of phosphorus by each species, efficiency use of phosphorus sum, assimilation of phosphorus by each species and assimilation of phosphorus sum. Pearson correlation coefficient was calculated to correlate diet offered per day with phosphorus concentration in water and sediment, and the significance of the correlation coefficient was assessed using *t* test (Sokal and Rohlf, 1995). Analyses were performed on SigmaStat for Windows (Systat Software Inc., version 3.5). The correlations and the graphs were performed on SigmaPlot for Windows (Systat Software Inc., version 11.0). The *t* test were performed on Microsoft Excel (Microsoft Corporation, version 14.0.4760.1000).

## **3. RESULTS**

No significant difference was shown for the phosphorus input compartments. Significant differences were shown for the outputs of harvested prawn and sediment (Table 2). Diet was the main source of phosphorus in all treatments, corresponding to 60.3% in LM, 63.4% in the LP and 64.0% in the LPC of all accounted compartments (Figure 1). Phosphorus input by rainwater was considered negligible at 0.32 kg/ha in all treatments, representing 0.5% of

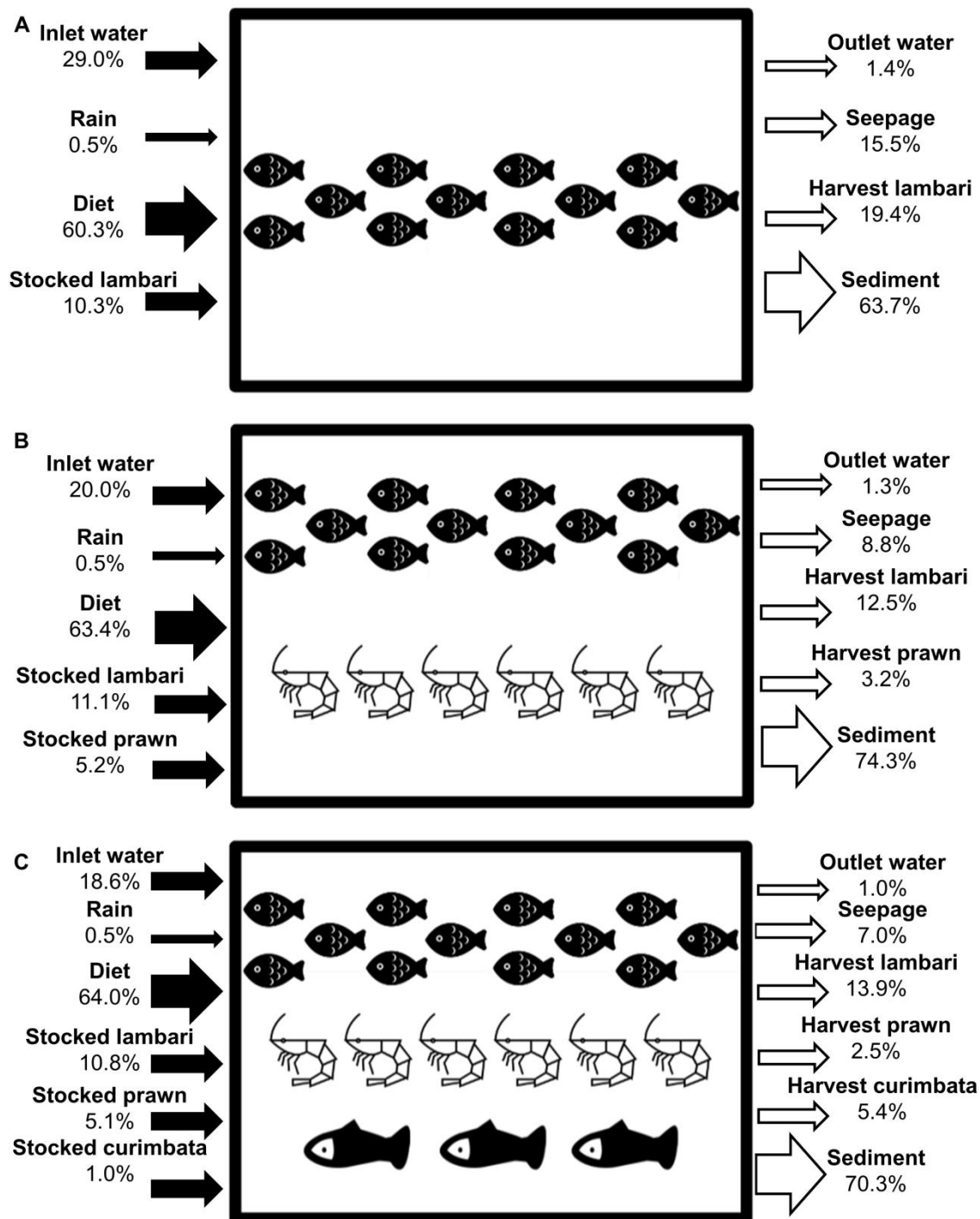
the sum of inputs of all accounted compartments. Sediment accumulated the most phosphorus in the ponds, corresponding to 63.5%, 74.1% and 70.1% in the treatments LM, LP and LPC, respectively. The outlet water represented the lowest phosphorus output, ranging from 1.0% to 1.4% for all treatments (Figures 1 A, B, C). Phosphorus output by sediment and harvest prawn was positively correlated with the addition of species (Figure 2 A and Figure 3). Sedimentation was positively correlated to the number of species in the ponds, being highest in the treatment LPC (Figure 2 B). The Unaccounted Portion (UP) was negative for all treatments, corresponding to 52.0% in LM, 69.2% in LP and 68.1% in LPC of input. The UP showed no statistical difference among treatments (Table 2).



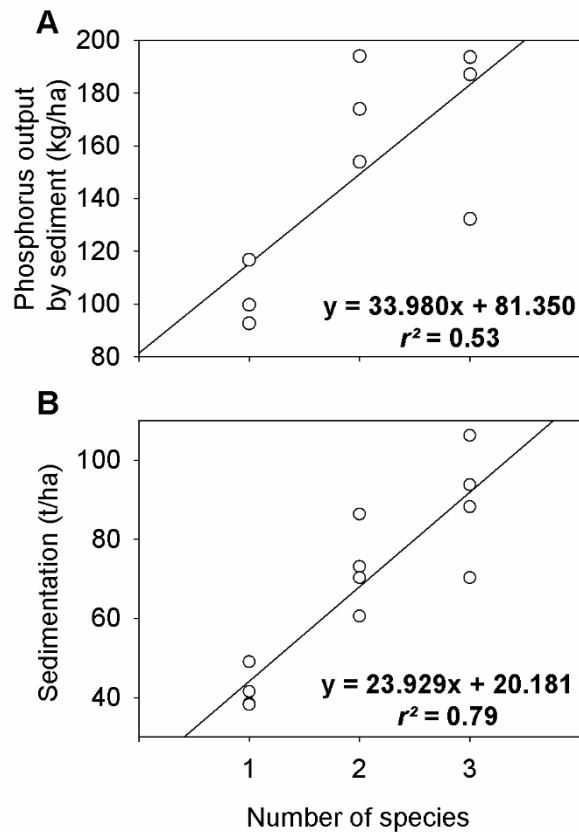
**Table 2.** Means ( $\pm$ SD) of the phosphorus loads in each pond compartment for the phosphorus budget of the monoculture of yellow tail lambari (*Astyanax lacustris*) (LM), integrated culture of yellow tail lambari and Amazon river prawn (*Macrobrachium amazonicum*) (LP) and integrated culture of yellow tail lambari, Amazon river prawn and curimbatá (*Prochilodus lineatus*) (LPC). Linear regression of the phosphorus content in each compartment against the number of farmed species was performed and equations obtained are presented when the coefficient of determination ( $r^2$ ) was higher than 0.25.

|                         | Treatments                 |                             |                            |       |                       |
|-------------------------|----------------------------|-----------------------------|----------------------------|-------|-----------------------|
| Ecological compartments | LM<br>(kg/ha)              | LP<br>(kg/ha)               | LPC<br>(kg/ha)             | $r^2$ | Equation              |
| Input                   |                            |                             |                            |       |                       |
| Inlet water             | 20.2 ± 6.3                 | 12.5 ± 1.5                  | 12.3 ± 4.1                 | 0.37  | y = -3.957x + 22.943  |
| Rainwater               | 0.3 ± 2.2.10 <sup>-5</sup> | 0.3 ± 2.2.10 <sup>-5</sup>  | 0.3 ± 2.1.10 <sup>-5</sup> | 0.01  |                       |
| Diet                    | 41.2 ± 2.7                 | 40.1 ± 2.6                  | 41.5 ± 3.2                 | 0.01  |                       |
| Stocked lambari         | 7.0 ± 2.8.10 <sup>-5</sup> | 7.0 ± 3.3.10 <sup>-5</sup>  | 7.0 ± 2.6.10 <sup>-5</sup> | 0.14  | y = -1.648x + 10.019  |
| Stocked prawn           | -                          | 3.3 ± 2.42.10 <sup>-5</sup> | 3.3 ± 2.5.10 <sup>-5</sup> | 0.00  |                       |
| Stocked curimbatá       | -                          | -                           | 0.7 ± 1.9.10 <sup>-5</sup> |       |                       |
| Total                   | 68.7 ± 8.1                 | 63.2 ± 3.1                  | 65.0 ± 6.4                 | 0.07  |                       |
| Output                  |                            |                             |                            |       |                       |
| Sediment                | 103.0 ± 12.4 <sup>a</sup>  | 173.9 ± 20.0 <sup>b</sup>   | 171.0 ± 33.7 <sup>b</sup>  | 0.53  | y = 33.980x + 81.350  |
| Outlet water            | 2.1 ± 0.9                  | 2.8 ± 1.5                   | 2.0 ± 0.3                  | 0.01  |                       |
| Seepage water           | 24.8 ± 18.2                | 19.4 ± 9.8                  | 15.3 ± 7.1                 | 0.11  |                       |
| Harvest lambari         | 28.2 ± 6.2                 | 26.0 ± 3.2                  | 27.2 ± 4.7                 | 0.01  | y = -1.648x + 10.019  |
| Harvest prawn           | -                          | 6.7 ± 1.0 <sup>a</sup>      | 5.1 ± 0.3 <sup>b</sup>     | 0.64  |                       |
| Harvest curimbatá       | -                          | -                           | 11.3 ± 2.6                 |       |                       |
| Total                   | 148.8 ± 42.2               | 213.8 ± 45.8                | 211.9 ± 54.4               | 0.25  | y = 31.536x + 128.450 |
| Unaccounted             |                            |                             |                            |       |                       |
| Input – Output          | -80.1 ± 36.0               | -150.6 ± 47.4               | -146.9 ± 49.0              | 0.29  | y = -33.376x – 59.098 |

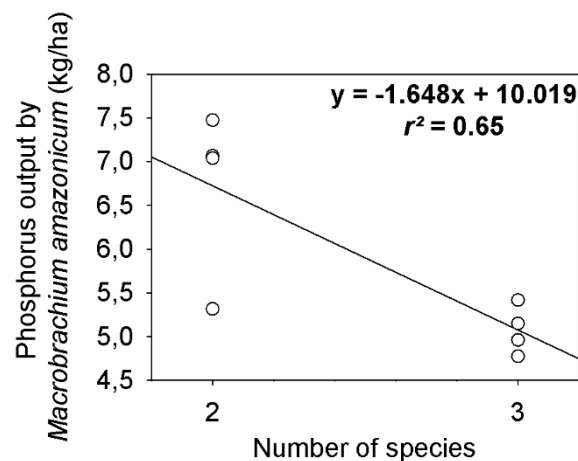
\* Means followed by different letters in the same line indicate significant difference between the treatments by ANOVA followed by Fisher-LSD test ( $P < 0.05$ )



**Figure 1.** Phosphorus budget in yellow tail lambari (*Astyanax lacustris*) monoculture (A), IMTA system with yellow tail lambari and Amazon river prawn (*Macrobrachium amazonicum*) (B) and IMTA system with yellow tail lambari, Amazon river prawn and curimbata (*Prochilodus lineatus*) (C). Values are shown in percentages based on the total input and output to pond.



**Figure 2.** Relation between number of farmed species and phosphorus output by sediment (A) and sedimentation (B) of yellow tail lambari (*Astyanax lacustris*) monoculture, IMTA system with yellow tail lambari and Amazon river prawn (*Macrobrachium amazonicum*) and IMTA system with yellow tail lambari, Amazon river prawn and curimbatá (*Prochilodus lineatus*).



**Figure 3.** Relation between number of farmed species and phosphorus output by Amazon river prawn (*Macrobrachium amazonicum*) of IMTA system with yellow tail lambari (*Astyanax lacustris*) and IMTA system with yellow tail lambari, Amazon river prawn and curimbatá (*Prochilodus lineatus*).

No statistical difference was shown among treatments in feed phosphorus conversion (FPC) and phosphorus use efficiency (PUE) by lambari. FPC values were nearby 50% and efficiency around 30% (Table 3). There is a statistical difference between prawn in treatments LP and LPC. The treatment LPC showed that curimbatá has an intermediate value of FPC and PUE, when compared to lambari and prawn, being almost half of the value presented by lambari in the same treatment. Both fishes were harvest with almost the same final mass. Number of species had a positive correlation with both FPC and PUE (Figure 4 A and B). Prawn has a low final mass, showing no difference between treatments LP and LPC. However, curimbatá presented an expressive final mass, then FPC and PUE sum in treatments LM and LP differ from LPC. Prawn showed high values of FPC and PUE in treatment LP, with a positive correlation between FPC and PUE by prawn, and the number of species (Figure 4 C and D).

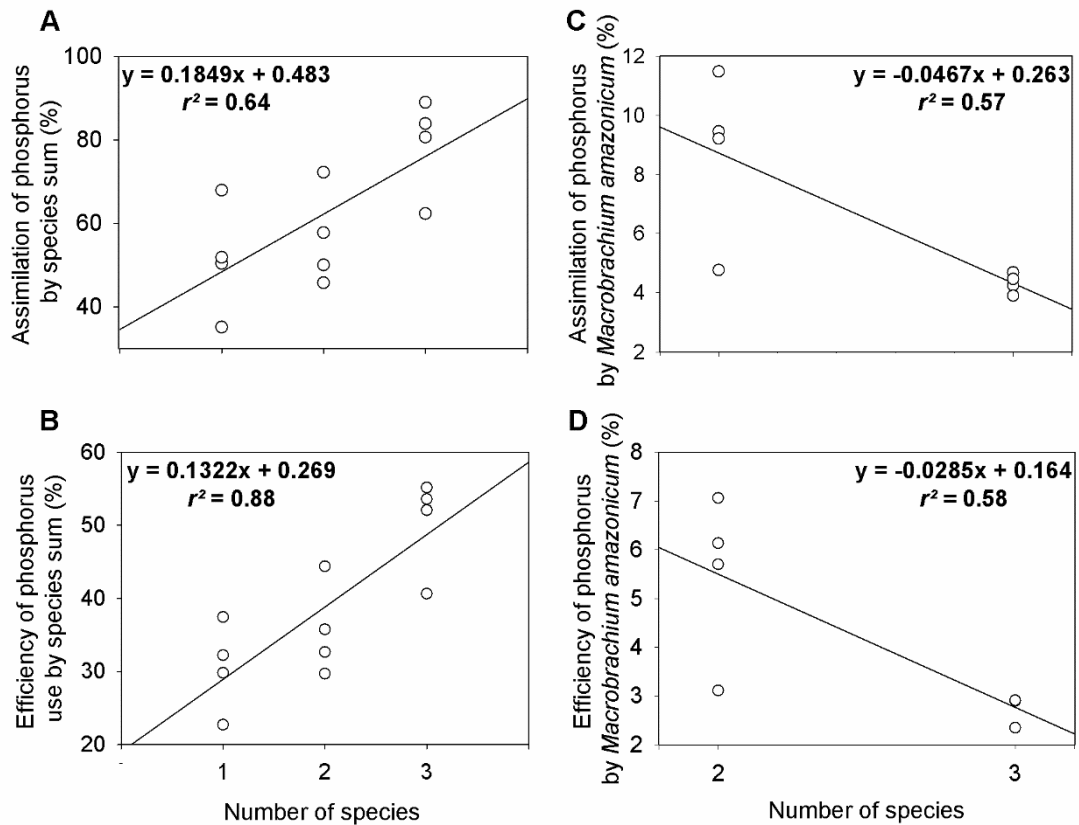
**Table 3.** Percent use efficiencies (mean  $\pm$  SD) of the phosphorus accumulated in the harvested biomass of yellow tail lambari (*Astyanax lacustris*), prawn (*Macrobrachium amazonicum*) and curimbatá (*Prochilodus lineatus*) in the treatments LM = monoculture of lambari, LP = integrated culture of lambari and prawn, LPC = integrated culture of lambari, prawn and curimbatá. Linear regression of the phosphorus incorporated by animals against the number of farmed species was performed and equations obtained are presented when the coefficient of determination ( $r^2$ ) was higher than 0.25.

| <b>Phosphorus incorporated by animals (%)</b> |                              |                              |                              |                         |                      |
|-----------------------------------------------|------------------------------|------------------------------|------------------------------|-------------------------|----------------------|
|                                               | <b>LM</b>                    | <b>LP</b>                    | <b>LPC</b>                   | <b><math>r^2</math></b> | <b>Equation</b>      |
| <b>FPC*</b>                                   |                              |                              |                              |                         |                      |
| <b>Lambari</b>                                | 51.3 $\pm$ 13.4              | 47.7 $\pm$ 10.1              | 49.1 $\pm$ 12.6              | 0.01                    | y = -0.0467x + 0.263 |
| <b>Prawn</b>                                  | -                            | 8.7 $\pm$ 2.8 <sup>a</sup>   | 4.3 $\pm$ 0.3 <sup>b</sup>   | 0.57                    |                      |
| <b>Curimbatá</b>                              | -                            | -                            | 25.5 $\pm$ 4.7               |                         |                      |
| <b>Total</b>                                  | 51.3 $\pm$ 13.4 <sup>a</sup> | 56.3 $\pm$ 11.6 <sup>a</sup> | 78.9 $\pm$ 11.6 <sup>b</sup> | 0.64                    | y = 0.185x + 0.483   |
| <b>PUE**</b>                                  |                              |                              |                              |                         |                      |
| <b>Lambari</b>                                | 30.5 $\pm$ 6.11              | 30.1 $\pm$ 5.64              | 31.4 $\pm$ 8.09              | 0.01                    | y = -0.0285x + 0.164 |
| <b>Prawn</b>                                  | -                            | 5.5 $\pm$ 1.7 <sup>a</sup>   | 2.8 $\pm$ 0.3 <sup>b</sup>   | 0.58                    |                      |
| <b>Curimbata</b>                              | -                            | -                            | 16.2 $\pm$ 2.5               |                         |                      |
| <b>Total</b>                                  | 30.5 $\pm$ 6.1 <sup>a</sup>  | 35.6 $\pm$ 6.3 <sup>a</sup>  | 50.4 $\pm$ 6.6 <sup>b</sup>  | 0.78                    | y = 0.132x + 0.269   |

\*The FPC (Feed Phosphorus Conversion) was measured as the proportion (%) of the harvested biomass phosphorus (kg/ha) over the phosphorus content of the commercial diet (kg/ha),

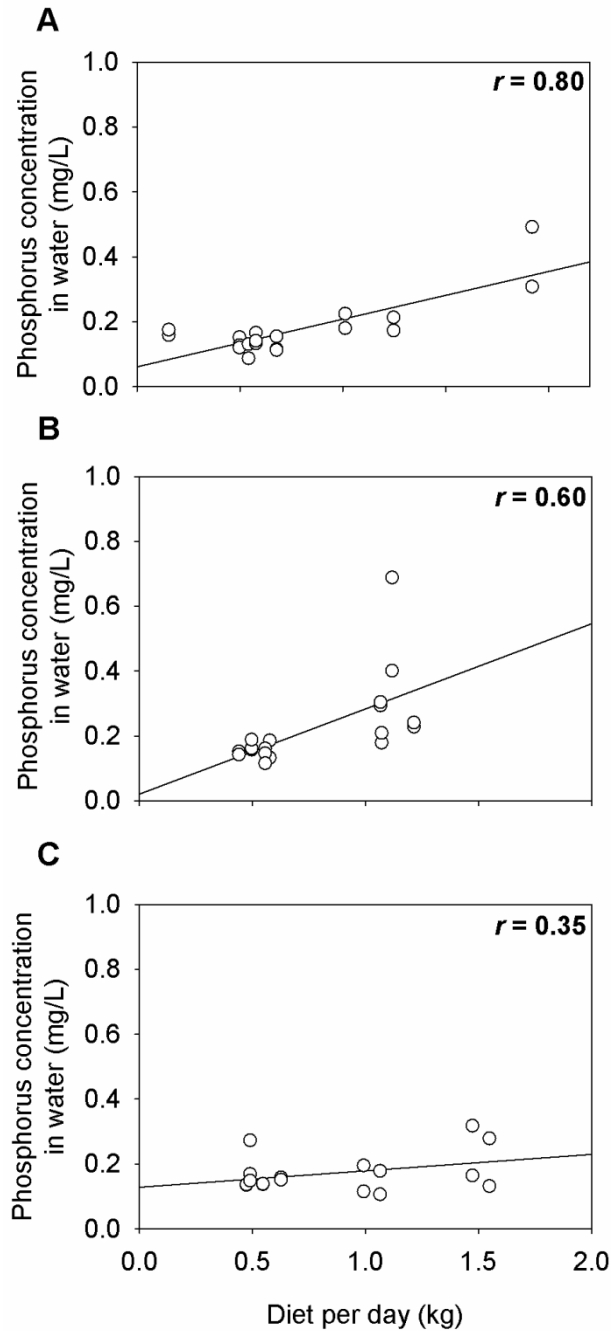
\*\*Environmental PUE (Phosphorus Use Efficiency - %) was considered to be the harvested biomass phosphorus (kg/ha) over the phosphorus from all inputs (kg/ha).

Means followed by different letters in the same line indicate significant difference between the treatments by ANOVA followed by Fisher-LSD test ( $P < 0.05$ ).



**Figure 4.** Relationship between number of species and assimilation of phosphorus by species sum (A), efficiency of phosphorus use by species sum (B), assimilation of phosphorus by Amazon river prawn (*Macrobrachium amazonicum*) (C) and efficiency of phosphorus by Amazon river prawn.

Phosphorus concentration in water showed a positive and significant correlation with diet offered by day in treatment LM ( $\alpha = 0.1\%$ ) (Figure 5 A), but there is a decrease in this correlation in treatment LP ( $\alpha = 5\%$ ) (Figure 5 B) and there is no significant correlation between these data in treatment LPC (Figure 5 C). There is no significant correlation between phosphorus concentration in sediment and diet offered by day in treatment LM,  $r = 0.16$ . There is a weak significant correlation in treatments LP,  $r = 0.56$ , and LPC,  $r = 0.60$  ( $\alpha = 5\%$ ).



**Figure 5.** Correlation between diet offered per day and phosphorus concentration in water of yellow tail lambari (*Astyanax lacustris*) monoculture (A), IMTA system with yellow tail lambari and Amazon river prawn (*Macrobrachium amazonicum*) (B) and IMTA system with yellow tail lambari, Amazon river prawn and curimatá (*Prochilodus lineatus*) (C).

#### 4. DISCUSSION

The integrated multitrophic aquaculture systems with two species, lambari and prawn, and three species, lambari, prawn and curimatá, had the same resources input as lambari monoculture. Monoculture produced 1.8

t/ha/cycle of lambari (Marques et al, 2021) and recovered 28.2 kg of P/ha/cycle, in other words, 15.6 kg of phosphorus for each tonne produced. The IMTA system of treatment LP produced 2.4 t/ha/cycle of lambari and prawn (Marques et al, 2021) and recovered 13.6 kilos of phosphorus by tonne produced. The IMTA system of treatment LPC produced 3.2 t/ha/cycle of lambari, prawn and curimbatá (Marques et al., 2021) and recovered 13.9 kg of phosphorus by tonne produced, considering total yield of each treatment. The IMTA system with tambaqui and Amazon river prawn recovered 2.5 kg of P/t produced (Flickinger et al., 2020), in an experiment carried by 171 days, and the IMTA system with tilapia and Amazon river prawn recovered 5.3 kg of P/t produced (David et al., 2017) in an experiment carried by 140 days. In treatment LP, prawn recovery almost twice phosphorus than in treatment LPC, demonstrating a satisfactory relation between lambari and prawn. In this study, prawn and curimbatá fed of bottom fauna, fish feces and unconsumed commercial diet, as was stated by New and Valenti (2017) for IMTA systems with tilapia. Previous studies were carried out with higher densities of Amazon river prawn (Dantas et al., 2020; Flickinger et al., 2020), lasted longer than the present study (David, 2017; Flickinger, 2020) and combine prawn with different species of fish, as tilapia and tambaqui (David, 2017; Dantas et al, 2020). It suggests that lower densities of unfed prawn, especially with lambari, are more adequate to the recovery of phosphorus from the others compartments of the ponds, although prawn recovery seems lower with curimbatá addition. Also, it indicates that species with shorter periods of growth as lambari can improve the recovery of phosphorus in short term, once that the initial recovery of phosphorus in the culture seems more intense.

Data variability decreased the power of the ANOVA test (Sokal and Rohlf, 1995), inhibiting the detection of differences among the systems. The high variability is probably because the experimental units are ponds in outdoor systems, constantly exposed to climate variations and external factors that cannot be controlled, as rain and temperature. Each pond has a different biota that can increase this variability.

The main phosphorus input source was the diet. It corresponded to 60.3 – 64.0% of the total input, followed by inlet water (18 – 29%) and lambari biomass (10.3 – 11.1%). These data are similar to Sahu et al. (2015), David et



al. (2017), Flickinger et al. (2020), that represented 93%, 50% and 75.1 of all input, respectively, showing that feed was the main phosphorus input. Furthermore, it is a daily input in system, with high kilograms values in the end of the production cycle. This huge amount of nutrient input through diet, that is nutrient-rich, can lead to the eutrophication of aquatic systems when not utilized (Cho et al., 1994; Boyd, 2020). A great portion of phosphorus was lost to sediment (63.7 – 74.3%), even though phosphorus concentration in sediment is not correlated to the diet offer by day in kilos. There is a positive correlation between phosphorus concentration in water and the diet offer by day, but this correlation decreases progressively whereas incorporation of phosphorus sum by animals increases with the addition of the other species. A closed IMTA system may decrease the pollutants caused by feeding on effluents, in comparison with recirculating water systems (Mongirdas et al., 2017), once that the addition of different species may improve the use of these nutrients, when available. Phosphorus was inefficiently used, because of the huge amount lost to the sediment or the seepage (7.0 – 15.5%) and the low proportion retained in animals (19.4 – 21.8%), when compared to other output compartments.

Sediment was the main output compartment, representing over half of phosphorus output (63.7% in LM, 74.3% in LP and 70.3% in LPC). Phosphorus content in samples was similar, around 20 mg/kg, but sedimentation increased with the addition of species, from 41.8 t/ha/cycle in treatment LM to 89.6 t/ha/cycle in treatment LPC. The sediment's capacity to retain phosphorus is very large, and it may present future saturation of this nutrient in the system (Boyd, 2020). In this case, aquaculture ponds may have phosphorus plots made available to other cultures. Combined bioturbation of prawn and curimbatá in the bottom of the pond resuspends the sediment, making phosphorus, nitrogen and other nutrients available in the water column as suggested by Adámek and Maršálek, 2013 (2020) and confirmed by Flickinger (2020). Phosphorus has low solubility, and thus, sediment rapidly (Flickinger et al., 2020; Boyd, 2020). Besides, the material accumulated in the bottom of the pond usually is mostly composed by dead phytoplankton and periphyton mats that are supplied by nutrient-rich diet wastes (Azim and Little, 2006). It may justify high content of phosphorus in sediment instead of outlet water that was the least representative

compartment, and showed only 1.0 – 1.4% of the phosphorus content, with no difference among treatments.

Phosphorus assimilated by animals from diet was approximately 80% in IMTA system with the three species and the efficient use of this nutrient, was 50.4%. When these values are compared to the other systems, that reached 56.3% and 35.6% in the treatment LP of assimilation and efficiency, respectively, and 51.3% and 30.5% in LM, it shows that, besides the high nutrients loss to the system in sediment or seepage, the treatment with three species seems effective in phosphorus uptake from the system and converted it into valued biomass. Phosphorus incorporation by curimbatá was high, once that this fish corresponded to 15.6% of total yield, but 32% of assimilation and efficiency of phosphorus use. These values are higher than those obtained by Flickinger et al. (2019), which assimilation was 23.6% and efficiency of use was 18.2% in IMTA system with tambaqui and Amazon river prawn. The phosphorus content in sediment was lower in Flickinger et al. (2020) study. Prawn has a benthic omnivorous feeding habit and curimbatá has an iliophagous feeding habit, and thus, both of them feed on the organic matter available at the bottom of the pond. Higher values of phosphorus in the sediment can be caused by dead phytoplankton and other organisms that reach the bottom of the pond by gravity. This availability of nutrients may offer more phosphorus to organisms, and it can improve the assimilation by the animals when compared to other studies. The assimilation of phosphorus by prawn was lower in the presence of curimbatá. It suggests that curimbatá can incorporate more available nutrients in the system as unconsumed diet and organic matter accumulated in sediment because of its iliophagous habit that resuspends particules and may become available the retained phosphorus of deeper layers of the pond. Prawn and curimbatá may compete for resources, once that both live in the same space and occupy similar feed niches. Prawn may be more selective than curimbatá, and do not incorporate phosphorus as well as in treatment LP, even though its final mass and length were similar in both treatments (Marques et al, 2021).

Inlet water represented 18 – 29% of phosphorus input, with no differences among treatments. David et al. (2017) reported similar values, corresponding to 17.2% (24.2 kg of P/ha), and Flickinger et al. (2020) presented 21.4% (15.0 kg of P/ha). On the other hand, a polyculture with three species of

Indian major carps *Catla catla* (Catla), *Labeo rohita* (Rohu), *Cirrhinus mrigala* (Mrigal) and a freshwater prawn (*Macrobrachium rosenbergii*) showed lower values, with inlet water representing 0.07% of phosphorus input (0.09 kg of P/ha) (Sahu et al., 2015). Inlet water includes the water used for flooding the ponds and to replace losses to evaporation and seepage. The water used in this study is nutrient-rich from a reservoir that receives aquaculture effluents. High data variability may be due to each pond particularity, as size and amount of water lost to seepage. Nutrient-rich water is suitable to aquaculture, even though it accumulates sediment in the bottom of the pond and carries materials that are not immediately biodegradable. It is a cost-free alternative to the addition of nutrients in water with fertilizers that is no longer necessary, and can be an economic benefit to production systems (Kimpapa et al., 2011).

Seepage represented 8.8 – 15.5% of phosphorus output, what corresponds to a loss of 0.9 – 1.8 million liters of water during production cycle. Similar results were observed by Flickinger et al. (2020) (0.9–1.7 million liters of water) that carried out the experiment in the same ponds. Sediment has favorable characteristics to phosphorus retention. New studies are necessary to confirm if phosphorus associated with seepage waters percolate to underground waters or is retained in the soil, which may act as a filter, decreasing the pollution of this production model. Outlet water was the least representative compartment in phosphorus output, representing 1.0 – 1.4% (2.0 – 2.9 kg/ha) of total output, meanwhile inlet water represented 18.6 – 29% (12.3 – 20.2 kg/ha). It reinforces the theory that nutrients are retained in sediment (Boyd, 2020; Flickinger et al., 2020), besides of the phosphorus incorporation by the organisms. All treatments suggest to be positive regarding to the use of phosphorus in water, once that inlet water was nutrient-rich and outlet water showed only 10% of initial content.

Rainwater content is a negligible compartment when compared to diet input (Boyd, 2020; Masuda and Boyd, 1994). In this study rainwater was the least representative input compartment (0.5% - 0.3 kg of P/ha). Similar data were reported by David et al. (0.3% - 0.5 kg of P/ha) (2017) and Flickinger et al. (0.4% - 0.3 kg of P/ha) (2020), indicating that phosphorus are not transferred to ponds by direct rain. However, nutrients as phosphorus can be carried to the ponds because of runoff processes (Boyd, 2020), which were not measured in

the present study It demonstrates the need of developing a new methodology that is able to collect a sample of the material that comes into the pond by runoff, aiming to calculate real rain impacts on nutrients budget.

Unaccounted portions (UP) had a high value in all treatments and are common in nutrient budgets, due to various factors such as methodological errors in determination of compartment contents and compartments that were not included in the study (David et al., 2017). UP were in the phosphorus input, showing values of 52.0 – 69.2%. The comparison among studies is difficult because of the particularity of each study about management, cultured species, and selected compartments to mass balance but Flickinger et al. (2020) and David et al. (2017) also showed higher values in phosphorus output of IMTA systems with a fish species and Amazon river prawn in cultures carried in the same ponds. Some compartments are difficult to quantify, such as the nutrients input by rain runoff, fallen leaves and flowers, trees that may be close to the pond and feces of aquatic birds. Besides that, phosphorus content in soil were not quantified before the experiment, so its presence in sediment output can be a consequence of nutrients input from previous experiments carried out in the same ponds. It could increase total phosphorus output and, consequently, input UPs. Although a large portion of the input was unaccounted, it was important to comprehend the nutrient flux in these systems.

## **5. CONCLUSION**

Phosphorus budgets analysis reveals that diet and sediment compartments are relevant input and output, respectively. Phosphorus assimilation by animals in IMTA system with three species is higher than in lambari monoculture, showing optimization of resource use. The addition of curimbatá increased the incorporation of phosphorus but decreased phosphorus incorporation by the prawn, suggesting that these animals compete for the resources of the same nutrients. However, it does not affect the viability of any species. Future research may be driven to study nutrients in soil and develop a new methodology that could quantify the input of nutrients by rainwater runoff. Despite species addition not show any statistical difference among treatments in phosphorus budget, all systems seem promising about reusing nutrients present in water.

## ACKNOWLEDGEMENTS

This research was funded by São Paulo Research Foundation–FAPESP, processes # 10/52210-3, 2018/15702-4, 2018/10363-7 and 2018/23993-9, National Council for Scientific and Technological Development–CNPq, processes # 406069/2012-3, 306361/2014-0, 140910/2018-2, 140911/2018-9 and 130870/2019-6, CAPES, process # 88882.433723/2019-01 and CAPES EMBRAPA public notice: 15/2014, project number 24; FINEP, agreement nº 01.10.0578.00/10.

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## **CAPÍTULO 3**

### **CONCLUSÕES GERAIS**

O balanço de fósforo foi importante para a compreensão da origem de entrada e saída desse nutriente e como é feita a compartimentalização do mesmo no sistema de monocultivo de lambari-do-rabo-amarelo, sistema integrado multitrófico de lambari-do-rabo-amarelo e camarão-da-amazônia e sistema integrado multitrófico de lambari-do-rabo-amarelo, camarão-da-amazônia e curimbatá. Observou-se que uma grande quantidade da entrada de fósforo no sistema não foi contabilizada, indicando a existência de outros compartimentos de entrada de fósforo que precisam ser pesquisados.

A entrada de fósforo pela dieta é bastante expressiva. Apesar disso, a alta incorporação de fósforo pelos animais é um fator positivo no que diz respeito ao aproveitamento do nutriente, principalmente por ser maior no cultivo com três espécies. A dieta geralmente é responsável por aumentar a concentração de fósforo em outros compartimentos, como sedimento e água. A escolha de espécies com funções ecológicas complementares foi fundamental para que existisse uma otimização do uso do fósforo nesses compartimentos, uma vez que os animais conseguiram incorporar quantidades expressivas do nutriente, diminuindo a oferta diária de alimento nas concentrações de fósforo na água com a adição de espécies.

A incorporação de fósforo mostra que existe uma diferença na assimilação do nutriente pelo camarão com e sem a presença do curimbatá. Mesmo tendo massa similar nos dois tratamentos, existe algum fator que diminui a eficiência do uso do fósforo pelo camarão. Estudos a respeito da nutrição e do comportamento das duas espécies são necessários para que se compreenda essa interação.

Para os próximos estudos, algumas metodologias precisam ser desenvolvidas, a fim de diminuir a parte não contabilizada do balanço, podendo então melhorar esse tipo de análise. A coleta do material lixiviado pela chuva e do sedimento antes do experimento pode ser importante para a compreensão do fluxo de entrada e saída do nutriente.

## CERTIFICADO DE APROVAÇÃO

Título: Balanço de fósforo no sistema de produção do lambari-do-rabo-amarelo em monocultivo e em cultivos integrados com camarão-da-amazônia e curimbatá

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