



The budget of carbon in the farming of the Amazon river prawn and tambaqui fish in earthen pond monoculture and integrated multitrophic systems

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ABSTRACT

The present study analyzed the inputs, outputs, and accumulation of carbon in ecological compartments of earthen ponds for the grow-out of Amazon river prawn (*Macrobrachium amazonicum*) and tambaqui fish (*Colossoma macropomum*) reared in monoculture and integrated multi-trophic aquaculture (IMTA) systems, using recycled hypereutrophic water. A completely randomized experiment was designed with four treatments and three replications: PM- monoculture with 30 prawn m⁻², FM- monoculture with 3 fish m⁻², IMTA- fish-prawn polyculture with 30 prawn m⁻² and 3 fish m⁻² reared free-swimming, POLY-CAGE- fish-prawn IMTA with 30 prawn m⁻² reared free-swimming and 40 fish m⁻³ in net-cages. Animals, rain, water, feed, soil, gases, total suspended solids, settleable solids, and accumulated sludge were collected throughout the experiment to determine their total organic and total inorganic carbon contents and to calculate the carbon budget. Results showed that much of the carbon accumulated as solid organic material (~55–84%) and animal biomass (~4–18%). The remaining carbon was discharged to receiving water bodies in the outlet water during harvesting (~6–8%) or emitted as carbon dioxide (CO₂) and methane (CH₄) (~1–5%). Feed management appeared to influence the major biological processes in the aquatic carbon cycle, such as photosynthesis and aerobic decomposition. All systems showed absorption of atmospheric CO₂ (~529–782 kg C-CO₂ ha⁻¹) that was higher than the emissions (~35–93 kg C-CO₂ ha⁻¹) and CH₄ (~14–123 kg C-CH₄ ha⁻¹). Furthermore, all of the culture processes in the present study removed ~1,000 to 2,000 kg Total Carbon ha⁻¹ from the inlet water, and thus, these systems can provide important ecosystem services. The high accumulation of carbon in the settleable solids (~69% of outputs) and the adequate water quality maintained in the IMTA demonstrate the resilience of this grow-out system to high allochthonous inputs of organic matter as feed. An increased prawn density or the addition of a mud-feeder species to the culture may enhance the incorporation of carbon from settleable solids into harvested biomass, improving the efficiency of the systems.

1. Introduction

The increasing global demand for aquatic protein sources has led to the development and diversification of aquaculture. The industrialization of the activity may increase the emissions of greenhouse gases and eutrophication of aquatic environments, mainly from the expansion and intensification of fed aquaculture systems (Lymer et al., 2016; Clark and Tilman, 2017; Ainsworth and Cowx, 2018). Artificial feeds often

account for the majority of nutrient inputs in aquaculture. Allochthonous feed can lead to inadequate rearing conditions because of the high accumulation of organic material, which depletes dissolved oxygen by increasing aerobic decomposition. The feed carbon converted into the biomass of the farmed species has been recorded as ~13% for fish (Avnimelech and Lacher, 1979; Boyd, 1985) and ~16% for marine shrimp (Boyd and Teichert-Coddington, 1995; Muthuwani and Kwei Lin, 1995; Lin and Nash, 1996; Páez-Osuna et al., 1997; Funge-Smith

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and Briggs, 1998; Lemonnier and Brizard, 1998; Martin et al., 1998; Ritvo et al., 1998; Páez-Osuna et al., 1999). Uneaten feed and wastes accumulate in the pond bottoms, which retain 25% of the feed organic carbon while the rest is converted to carbon dioxide (CO₂) by aerobic processes (Avnimelech and Ritvo, 2003). Dissolved CO₂ enhances the primary production and allows the expansion of the aquatic food web, which leads to an increase in phytoplankton turnover (Moriarty, 1997; Boyd et al., 2010). Dead phytoplankton and other organic material return to the bottom sediments where they are subject to anaerobic decomposition, having the potential to cause the release of harmful metabolites.

Earthen ponds can assimilate and transform nutrients through the mineralization of organic matter, liberating nutrients to organisms of the aquatic food web, which would immobilize pollutants and maintain stable environmental conditions (Boyd et al., 2010; Kimpara et al., 2013). The organic carbon that accumulates in earthen pond systems may be assimilated by another species that has a complementary ecosystem function in relation to the target species (Chopin et al., 2012; Marques et al., 2016). Thus, the integration of an unfed species in intensively-fed monoculture fish farming may mitigate the accumulation of organic carbon by consuming nutrients in settled feed leftovers and wastes from the fish, thereby reducing the onset of adverse conditions within the pond as well as the surrounding environment that receives the effluents.

The knowledge of carbon budgets is necessary to the understanding of how carbon accumulates in earthen pond systems and how the target species may convert this key nutrient into harvested biomass (Liu et al., 2014; Mariscal-Lagarda and Páez-Osuna, 2014; Marques et al., 2016). Mass balances have been established for nitrogen and phosphorus in monocultures of some fish (Boyd, 1985; Daniels and Boyd, 1989; Green and Boyd, 1995) and shrimp species (Briggs and Funge-Smith, 1994; Hopkins et al., 1994; Páez-Osuna et al., 1997; Martin et al., 1998; Jackson et al., 2003; Thakur and Lin, 2003; Wahab et al., 2003; Casillas-Hernández et al., 2006; Sahu et al., 2013; Saraswathy et al., 2013; Adhikari et al., 2014). David et al. (2017a, b) analyzed nitrogen and phosphorus budgets in the grow-out IMTA of Nile tilapia *Oreochromis niloticus* and the Amazon river prawn *Macrobrachium amazonicum*. Flickinger et al. (2019; 2020) compared nitrogen and phosphorus budgets in the monocultures and IMTA of the tambaqui *Colossoma macropomum* and *M. amazonicum*. Some studies have reported on the carbon budget based on the organic carbon content of pond compartments in giant river prawn *Macrobrachium rosenbergii* monocultures (Sahu et al., 2013; Adhikari et al., 2014), in IMTA systems of Indian major carp and *M. rosenbergii* (Sahu et al., 2015) and raceway IMTA of various fish species (Brown et al., 2012). No study has compared the carbon budgets of freshwater IMTA to those of the monocultures of the same species with equal stocking densities. In addition, the available data do not include direct measures of the carbon greenhouse gases exchanged between the earthen pond system and the atmosphere.

The sustainability of aquaculture may be enhanced by rearing native species in integrated systems. This strategy improves the use of space, water, nutrients, and energy, in addition to avoiding the accidental release of exotic species and their symbiont organisms into the surrounding environment. The South American species *C. macropomum* and *M. amazonicum* can be farmed together in IMTA. The *C. macropomum* is pelagic and eats zooplankton, insects, fruits, and seeds (Araujo-Lima and Goulding, 1997). This species has been widely farmed in many South American countries such as Brazil, Colombia, Venezuela, and Peru. In Amazonia, the grow-out is frequently divided into two subsequent phases of ~5 – 6 months in ponds or net-cages (Wojnárovich and Van Anrooy, 2019). The *M. amazonicum* is epibenthic and feeds mainly on detritus and benthic organisms (Moraes-Valenti and Valenti, 2010). This prawn reaches market size after four to six months of grow-out in ponds (Moraes-Valenti and Valenti, 2007; Marques and Moraes-Valenti, 2012). Thus, the different spaces and

trophic levels occupied by the two species allow their aquaculture in the same earthen pond system. Recently, it was demonstrated that they do not show any negative interaction inside earthen aquaculture ponds (Dantas et al., 2020).

The objective of this study was to establish the carbon budgets for the monocultures of the *M. amazonicum* and the *C. macropomum*, and for the integrated culture of the two species during the first phase (first six months) of the *C. macropomum* grow-out. The systems were compared to each other by quantifying the total organic carbon, total inorganic carbon, and the carbon gas content in the ecological compartments of earthen ponds.

2. Materials and Methods

The experiment was performed in 12 earthen ponds, each with a surface of ~120 m² and 1 m water depth. They were built on eutrofic red latosol and are located in the Aquaculture Center, São Paulo State University (UNESP), Jaboticabal, Brazil (21°15'22"S, 48°18'48"W). A completely randomized design with four treatments and three replications of each were used. The type of production system was the factor tested with four levels: (1) prawn monoculture (PM); (2) fish monoculture (FM); (3) fish-prawn IMTA with the fish reared free-swimming (IMTA); (4) fish-prawn IMTA with the fish reared in net cages (POLY-CAGE). The experiment lasted 171 days, from November of 2013 to April of 2014. The procedures used were approved by the Ethics Committee on the Use of Animals (CEUA) of the School of Agronomy and Veterinary Sciences, UNESP (protocol no. 026984/11).

2.1. Pond Management

The leftover sediment from the previous experiments was removed from each pond, and agricultural lime was spread at a rate of 0.1 kg m⁻². One net cage was set up in each pond of the POLY-CAGE treatment. Net cages had a surface of 4 m² and a height of 1 m and were constructed of an 8 mm mesh. The submerged depth was 0.75 m, and therefore the volume was 3 m³. The ponds were filled using nutrient-rich water from a reservoir that receives effluents from frog and fish culture. Inflow water was subjected to mechanical filtration through a 1 mm mesh. Nets were set up over the ponds to avoid mitigate predation by birds. Water was not exchanged during the culture but was added to replenish water lost to evaporation and seepage. Total water loss was estimated biweekly by measuring the change in water depth for 12 hours with no added water.

Juveniles of the *M. amazonicum* (0.04 ± 0.01 g) were stocked at a density of 30 prawn m⁻² (300,000 prawn ha⁻¹) for the PM, IMTA, and POLY-CAGE treatments. The prawn were produced at the Aquaculture Center of São Paulo State University from broodstock caught in the Amazonia (01°14'30"S, 48°19'52"W). The prawns were stocked two weeks before the tambaqui fingerlings as a precautionary management strategy to avoid the predation of the prawns by the fish (Santos and Valenti, 2002). The tambaqui fingerlings (1.77 ± 0.71 g) were stocked at 3 fish m⁻² (360 fish in each pond) as free-swimming in the monoculture and IMTA treatments, and 40 fish m⁻³ (30 fish m⁻²; 120 fish in each pond) in the cages of the POLY-CAGE treatment. The fish were obtained from a farm in Amazonia (2°29'S, 59°37'23"W).

The feeding regime for all treatments began once the *C. macropomum* were stocked. Each month, 50 prawn and 20 fish were randomly sampled from each pond and weighed to recalculate the daily feeding rate using a mass balance with a precision of 0.01 g (Marte, model AS2000C, Brazil). In monoculture, the prawn were fed with an extruded marine-shrimp diet (Guabi Potimar, 38 – 40% crude protein, Brazil). The initial feeding rate was 10% of the total prawn biomass, reduced to 5% on day 97, and finalized at 3% on day 125. In the IMTA and POLY-CAGE treatments, prawn did not receive a commercial diet but fed on natural biota and fish wastes. In the fish monoculture, IMTA, and POLY-CAGE treatments, the daily feeding rate was adjusted based only on the

fish biomass. A commercial diet for omnivorous fish (Nutreco, Tilapia Starter, 45% crude protein, Brazil) was supplied at 6% of the fish biomass until they attained a mean individual mass of 100 g (day 97). Then, a commercial diet for omnivorous fishes (Nutreco Fri-Aqua for juveniles and Fri-Aqua for omnivorous species, 32% crude protein, Brazil) was used at 3% of the total fish biomass until the end of the experiment. The commercial diet was dispersed manually twice daily at 10:00 and 16:30 hours.

2.2. Pond water quality

Water replenishment varied between a minimum of 84.2% for the IMTA and a maximum of 91.3% for the PM treatment. The main variables of freshwater pond water quality were monitored. The following were measured daily *in situ* at 20 – 30 cm below the water surface at 07:00 h (Morning: M) and 16:00 h (Afternoon: A), using a YSI Professional Plus digital meter (Yellow Springs Instruments, Yellow Springs, OH, USA): temperature (M: 27.4 – 27.8 °C; A: 30.0 – 30.7 °C), dissolved oxygen (M: 4.3 – 5.3 mg L⁻¹; A: 8.8 – 12.0 mg L⁻¹), oxygen saturation (M: 58.7 – 72%; A: 129 – 167%), pH (M: 8.0 – 8.2; A: 9.1 – 9.7), and conductivity (M: 128 – 134; A: 140 – 145 μS cm⁻²). Total nitrogen (TN; Elementar-Vario TOC-N Select, Germany), total ammonia nitrogen (TAN; Koroleff, 1976; Mackereth et al., 1978), nitrite (NO₂⁻; Koroleff, 1976; Mackereth et al., 1978), total phosphorus (TP; APHA (American Public Health Association), 2005, 4500-P B5; APHA, 2005, 4500-P D), chlorophyll-*a* (Cla; Marker et al., 1978; Sartory and Grobbelaar, 1984), and transparency (T; Boyd, 2015) were measured biweekly throughout the experimental period. Ranges of each variable were: TN, 2.6 – 2.7 mg L⁻¹; TAN, 6.9 – 18.3 μg L⁻¹; NO₂⁻, 4.0 – 10.2 μg L⁻¹; TP, 0.19 – 0.24 mg L⁻¹; Cla, 0.14 – 0.21 μg L⁻¹; and T, 33 – 41 cm. Ranges of the water quality parameters obtained in the present study were similar to those previously observed for the monocultures of *M. amazonicum* (Moraes-Valenti and Valenti, 2007; Preto et al., 2011) and *C. macropomum* (Ferrari et al., 1991; Gomes et al., 2006; Cunha and Santos Junior, 2011). In addition, these parameters were within the recommendations described in Boyd (2015) for general freshwater aquaculture in earthen ponds.

2.3. Harvest

The experiment lasted 171 days. Then, the ponds were drained, and all fish and prawn were harvested and counted. All the fish and a random sample of 10% of the prawn from each pond were weighed (Marte AS2000C precision balance, São Paulo, Brazil; 0.1 g precision). Mean final individual mass, survival, and yield of the prawn were 2.6 – 5.0 g animal⁻¹, 63.7 – 75.1%, and 0.55 – 0.95 t ha⁻¹, respectively. For the fish, they were 129 – 181 g animal⁻¹, 92.5 – 96.6%, and 3.0 – 5.3 t ha⁻¹, respectively. The growth and survival rates of the Amazon river prawn were similar to those observed in Moraes-Valenti and Valenti (2007), and those of the tambaqui were similar to those observed in Gomes et al. (2006) and Cunha and Santos (2011). Total yield at harvest was approximately 1.0, 5.3, 5.0, and 3.6 t ha⁻¹ for the PM, FM, IMTA, and POLY-CAGE treatments, respectively. The productive performance of these species in the present study are detailed in Dantas et al. (2020).

2.4. Carbon budget

The carbon content in the main ecological compartments of the system were analyzed. The unaccounted portion (*UC*) was determined by the difference between the total carbon input (*TCin*) and the total carbon output (*TCout*). The equations used were:

$$TCin = IW + RW + CD + SF + SP + A-CO_2 + A-CH_4 \quad (1)$$

$$TCout = OW + HF + HP + TSS + SS + AS + D-CO_2 + D-CH_4 + E-$$

$$CO_2 + E-CH_4 \quad (2)$$

$$TCin - TCout = UC \quad (3)$$

in which *IW* (inlet water), *RW* (rainwater), *CD* (commercial diet), *SF* (stocked fish), *SP* (stocked prawns), *A-CO₂* (carbon dioxide gas absorption), and *A-CH₄* (methane gas absorption) refer to the carbon contents of the input compartments, and *OW* (outlet water), *HF* (harvested fish), *HP* (harvested prawn), *TSS* (total suspended solids), *SS* (settleable solids), *AS* (accumulated sludge), *D-CO₂* (carbon dioxide gas emission by diffusion), *D-CH₄* (methane gas emission by diffusion), *E-CO₂* (carbon dioxide gas emission by ebullition), and *E-CH₄* (methane gas emission by ebullition) refer to the carbon contents of the output compartments. Total carbon of the inlet water, outlet water, the sum of all inputs and the sum of all outputs are reported as kg C-TC ha⁻¹; organic and inorganic carbon loads of the water are reported as kg C-TOC ha⁻¹ and kg C-TIC ha⁻¹, respectively; organic carbon contents of solid material as kg C-TOC ha⁻¹, and carbon dioxide and methane gases as kg C-CO₂ ha⁻¹ and kg C-CH₄ ha⁻¹, respectively. The carbon assimilated by the animals was measured as the carbon retained from the commercial diet (feed carbon conversion - FCC) and the use efficiency of carbon from all inputs (carbon use efficiency - CUE).

Total organic carbon (TOC) and total inorganic carbon (TIC) input by inlet water and output by outlet water during the harvest were calculated by multiplying the total carbon concentrations by the total inlet and outlet water volume, respectively. The inlet water volume is the sum of water used to fill the ponds and the water added to compensate for loss from evaporation and seepage. The TOC (dissolved + particulate) and the TIC (dissolved inorganic carbon: the sum of the carbonates, bicarbonates, and carbonic gas) contents of the water were determined by oxidation catalytic combustion (Elementar – Vario TOC Select, Germany). The total carbon concentrations (TC = TOC + TIC) in the inlet water were measured at the pond flooding, at the beginning of the integrated culture, when fish were stocked, and biweekly during the replenishment of the water. The TC concentrations in the outlet water were measured on the drained water at the harvest. Rainfall data from the UNESP Agrometeorological Station, Jaboticabal was used to calculate the total precipitation volume during the culture period (measured in L m⁻²). These data were adjusted for each pond surface and then multiplied by the mean TC concentration in rainwater.

The mean total organic carbon concentration in the diets were determined using an elemental CHNS analyzer (Elementar – Vario MACRO Cube, Germany). These values were multiplied by the total amount of the diets supplied to obtain the input of carbon through the feed. Total organic carbon concentrations of stocked and harvested animals were obtained by samples analyzed in triplicate after drying, using an elemental CHNS analyzer (Elementar – Vario MACRO Cube Analyser, Germany). These values were multiplied by the total biomass of the animals to obtain the content of carbon in the farmed animals.

Solid materials were separated into five categories (Flickinger et al., 2019). Total suspended solids are the solid material suspended high in the water column. Total suspended solids represent phytoplankton, bacteria, aggregates of living and dead particulate organic matter, and grazers of the bacteria (Hargreaves, 2006). The settleable solids were the flocculent organic material that accumulates on the pond bottom, which is easily resuspended and drains with the harvested water (Teichert-Coddington et al., 1999). The settleable solids represent dead plankton, uneaten feed, culture animal waste, and other organic material (Boyd, 1995). There is a continuum in nutrient contents from the original bottom soil to the sludge, in which the sludge is comprised of soft, fluidized, organic-rich residue (Avnimelech and Ritvo, 2003). The accumulated sludge represents the compacted layer of settleable solids above the original bottom soil that is not drained with the harvested water. The bottom soil is the original soil that the ponds were constructed on, excluding the top centimeter that is removed by scraping

before filling the ponds. The term “bottom sediments” refers to all solid material that composes the pond bottoms, including the settleable solids, accumulated sludge, and bottom soil.

The total suspended solids (TSS) were analyzed biweekly for 100 mL of the culture water and for 100 mL of the effluents at the harvest (APHA (American Public Health Association), 2005; TSSs dried at 103–105 °C). The total organic carbon concentration of the settleable solids (SS) was used to determine the carbon content of the total suspended solids, which was then multiplied by the pond volume to determine the carbon accumulated as total suspended solids. Tripton samples were obtained biweekly using the methodology described in Flickinger et al. (2019). Samples were dried (AOAC (Association of Official Analytical Chemists), 1995; 934.01), weighed, and analyzed to determine the total organic carbon content using an elemental CHNS analyzer (Elementar – Vario MACRO Cube, Germany). The settleable solids mass and the total organic carbon concentration in the samples were used to estimate the total carbon of the settleable solids that accumulated on the pond bottoms over 24 hours. The output was determined with the final sample of the settleable solids before the harvest. The final sample of the settleable solids material that accumulated over 24 hours and the mean total organic carbon concentration of the settleable solids throughout the experiment were determined to characterize the bottom sediments (Table 1).

Samples of the bottom soil were taken before the ponds were filled. The accumulated sludge after harvest was determined using five 0.2 m² ceramic tiles placed inside the ponds before they were filled. The height of the accumulated sludge after the harvest was measured (cm). Samples of soil and sludge were dried (AOAC (Association of Official Analytical Chemists), 1995; 934.01), weighed, and analyzed to determine the total organic carbon content using an elemental CHNS analyzer (Elementar – Vario MACRO Cube, Germany). The accumulated sludge after harvest and the total organic carbon concentration in the samples were used to estimate the total amount of carbon that settled above the bottom soil throughout the culture. Characteristics of the bottom soil and accumulated sludge were determined to describe the bottom sediments of the ponds (Table 1).

Carbon dioxide (CO₂) and methane (CH₄) absorption from the atmosphere and emission were estimated biweekly by diffusion and bubbling (Matvienko et al., 2000). Diffusion at the air-water interface was measured using a diffusion chamber during the day (between 14:00–16:00 hours) and at night (between 22:00–24:00 hours). Fiberglass funnels suspended by floats were placed on the surface of the ponds to capture gas bubbles emitted over 24 hours. The gas samples from both methods were collected in transfer tubes for analysis by gas chromatography (Shimadzu Instruments – GC-2014 Permanent Gas Analyzer, Japan) with TCD (Thermal Conductivity Detector) and FID (Flame Ionizer Detector). The methods of collecting the gas samples and analyses are detailed in Flickinger et al. (2019)

Table 1

Means (± SD) of the various measurements related to the bottom soils for the grow-out of the Amazon river prawn (*Macrobrachium amazonicum*) and the tambaqui fish (*Colossoma macropomum*) in monoculture systems (Prawn Monoculture - PM and Fish Monoculture - FM), and in fish-prawn IMTA systems with the tambaqui reared as free-swimming (IMTA) and in cages (POLY-CAGE).

Parameters	Treatments			
	PM	FM	IMTA	POLY-CAGE
Pre-flood soil carbon (g C-TOC kg ⁻¹)	33.8 ± 6.4	32.0 ± 4.3	30.8 ± 3.2	20.9 ± 4.2
Accumulated sludge height (cm)	1.2 ± 0.4	1.1 ± 0.2	1.6 ± 1.0	1.0 ± 0.4
Accumulated sludge (kg ha ⁻¹)	45,501 ± 12,728	22,368 ± 19,480	29,251 ± 18,859	34,938 ± 23,992
Accumulated sludge carbon (g C-TOC kg ⁻¹)	45.0 ± 18.9	52.9 ± 16.9	43.3 ± 19.0	45.4 ± 32.0
Settleable solids (kg SS ha ⁻¹ day ⁻¹)	745 ± 341 b	830 ± 142 b	3,541 ± 1,445 a	1,206 ± 123 b
Settleable solids carbon (g C-TOC kg ⁻¹)	134.7 ± 29.0	129.6 ± 4.3	133.3 ± 9.0	132.3 ± 19.9

Note. Means followed by different letters in the same line indicate significant differences according to the Kruskal-Wallis test followed by the Wilcoxon rank-sum test.

2.5. Data analysis

The proportion of organic carbon from the commercial diet and total organic carbon from all sources converted into prawn and fish biomass were square-root arcsine-transformed before analysis. All data were tested for normality (Shapiro-Wilk test) and homoscedasticity (Levene test). When both conditions were satisfied, data were subjected to one-way ANOVA (*F*-test). When significant differences were detected among treatments, the means were compared post-hoc with the Tukey test. The organic carbon content of the settleable solids as an output, organic carbon from the commercial diet and carbon from all inputs converted into biomass, and three sampling periods for the daily sedimentation of settleable solids were not normal and were subjected to the Kruskal–Wallis test followed by the Wilcoxon rank-sum test. The analyses were carried out using the Statistical Analysis System (SAS Institute Inc., version 9.0), and the level of significance considered was $\alpha = 0.05$.

Diagrams were assembled, showing the carbon content in each compartment of each system and the flow of carbon in each system. Pearson’s correlation coefficient was calculated to determine the strength of the linear correlation between several variables. All values determined for each pond were entered into the correlation analyses, and the significance of the correlation coefficient was assessed using the *t*-test (Sokal and Rohlf, 1995). The correlation analyses were carried out in the R software version 0.98.945 (R Foundation for Statistical Computing, Vienna, Austria), and the level of significance considered was $\alpha = 0.05$.

3. Results

Significant differences in the characteristics of the pond bottom were only shown for the daily sedimentation rate of the settleable solids (Table 1). The settleable solid material was significantly higher in the IMTA when compared to the other treatments. The carbon budgets for each ecological compartment was converted in kg C ha⁻¹ and mapped as percentage flows for the inputs and outputs (Table 2 and Fig. 1). The input compartment of the diet was similar for the FM and IMTA treatments, both of which were significantly higher than the PM and POLY-CAGE. The feed carbon input for the PM and IMTA-CAGE treatments were lower due to having lower biomass inside the ponds. Inlet water, diet, and absorption of carbon dioxide were the major carbon inputs for all treatments. The inlet water accounted for approximately 56%, 41%, 18%, and 49% of input carbon, and the diet accounted for approximately 24%, 47%, 23%, and 11% for the PM, FM, IMTA, and POLY-CAGE treatments, respectively. The total organic carbon and total inorganic carbon fractions of the inlet water represented ~7–21% and ~12–36%, respectively, of all carbon inputs. Carbon dioxide absorbed from the atmosphere ranged from approximately 9 to 19% of the inputs. The remaining inputs (rainwater, stocked animals, and methane absorption) contributed approximately 1% or less of the carbon.

Table 2

Means (\pm SD) of the carbon loads in each pond compartment for the carbon budget of the Amazon river prawn (*Macrobrachium amazonicum*) and the tambaqui fish (*Colossoma macropomum*) grow-outs reared in monoculture systems (Prawn Monoculture - PM and Fish Monoculture - FM), and in fish-prawn IMTA systems with the tambaqui reared as free-swimming (IMTA) and in cages (POLY-CAGE).

Ecological compartments (kg C ha ⁻¹)		Treatments			
		PM	FM	IMTA	POLY-CAGE
Inputs					
Inlet water (total carbon)		2,335.1 \pm 686.2	1,979.6 \pm 307.1	1,437.7 \pm 414.7	1,832.4 \pm 371.2
Total organic carbon		854.0 \pm 235.8	730.0 \pm 116.1	522.9 \pm 156.2	686.5 \pm 161.6
Total inorganic carbon		1,481.1 \pm 452.1	1,249.6 \pm 198.3	914.8 \pm 258.5	1,145.9 \pm 210.2
Rainwater		32.5 \pm 5.3E-3	32.5 \pm 7.4E-3	32.5 \pm 8.1E-3	32.5 \pm 9.5E-3
Commercial diet		993.6 \pm 199.7 b	2,297.8 \pm 325.4 a	1,798.6 \pm 325.4 a	392.0 \pm 52.9 b
Stocked fish			6.2 \pm 1.8E-3 b	6.2 \pm 4.9E-3 b	1.8 \pm 0.2 a
Stocked prawns		0.8 \pm 1.4E-16		0.8 \pm 1.8E-16	0.8 \pm 1.6E-16
Gas absorption:					
CO ₂		781.6 \pm 159.5	529.4 \pm 93.6	682.9 \pm 92.9	594.0 \pm 60.4
CH ₄		4.9 \pm 3.2	8.3 \pm 4.1	27.0 \pm 38.1	5.5 \pm 2.8
Outputs					
Outlet water (total carbon)		328.3 \pm 63.4	336.3 \pm 71.8	452.9 \pm 113.0	291.6 \pm 63.8
Total organic carbon		211.7 \pm 69.1	230.4 \pm 54.8	288.8 \pm 105.0	181.3 \pm 42.1
Total inorganic carbon		116.5 \pm 12.2 b	105.9 \pm 19.6 b	164.2 \pm 9.4 a	110.3 \pm 21.9 b
Harvested fish			853.1 \pm 203.3 a	571.7 \pm 98.9 a	114.6 \pm 33.0 b
Harvested prawns		152.0 \pm 30.5 a		91.6 \pm 14.4 b	77.3 \pm 23.5 b
Total suspended solids		85.8 \pm 46.0	76.7 \pm 30.4	155.4 \pm 62.4	131.5 \pm 88.2
Settleable solids		1,079.1 \pm 197.8 b	1,476.7 \pm 124.9 b	5,390.3 \pm 2,678.7 a	1,789.3 \pm 101.1 b
Accumulated sludge		1,930.9 \pm 453.5	1,101.0 \pm 785.0	1,027.7 \pm 441.9	1,139.1 \pm 379.3
Gas diffusion:					
CO ₂		31.7 \pm 13.5	67.6 \pm 29.4	74.3 \pm 35.6	68.3 \pm 54.3
CH ₄		3.6 \pm 2.7	2.3 \pm 2.1	2.5 \pm 2.5	0.8 \pm 0.9
Gas ebullition:					
CO ₂		2.9 \pm 1.7 b	18.0 \pm 6.6 ab	19.1 \pm 13.8 a	8.4 \pm 5.6 ab
CH ₄		14.7 \pm 18.3	12.0 \pm 16.2	44.8 \pm 26.4	122.3 \pm 123.9
Unaccounted carbon:					
Inputs - Outputs		519 \pm 1,246	910 \pm 678	-3,845 \pm 3,440	-884 \pm 849

Note. Total organic carbon and total inorganic carbon describe the composition of the total carbon in the inlet water and outlet water compartments and were not included when calculating the sum of inputs and outputs for each treatment. Means followed by different letters in the same line indicate a significant difference between the treatments by ANOVA followed by the Tukey test ($P < 0.05$).

Most of the carbon was drained from the earthen ponds with the outlet water (~6 – 8%) and retained in the settleable solids (~26 – 69%) and accumulated sludge (~13 – 47%), the sum of which (OW + SS + AS) ranged from approximately 60 to 88% (Fig. 1). The carbon of the harvested biomass ranged from ~3 – 18% in fish and ~1 – 4% in prawn. The carbon in the *C. macropomum* biomass was similar between the FM (853.1 \pm 203.3 kg C-TOC ha⁻¹) and IMTA (571.7 \pm 98.9 kg C-TOC ha⁻¹) treatments, both of which were significantly higher than the POLY-CAGE treatment (114.6 \pm 33.0 kg C-TOC ha⁻¹), in which the number of fish stocked per pond was ~3 times lower. The carbon in the prawn biomass of the PM (152.0 \pm 30.5 kg C-TOC ha⁻¹) was significantly higher than those of the IMTA (91.6 \pm 14.4 kg C-TOC ha⁻¹) and POLY-CAGE treatments (77.3 \pm 23.5 kg C-TOC ha⁻¹). Each treatment showed a higher CO₂ gas absorption than emission, and atmospheric CO₂ was shown to enter the earthen ponds throughout the experimental period (Fig. 2).

The budget showed positive values for the monocultures ($TC_{in} > TC_{out}$) and negative values for the IMTA and POLY-CAGE treatments ($TC_{in} < TC_{out}$) (Table 2 and Fig. 1). The highest proportion of unaccounted carbon was found in the IMTA treatment at ~49% of the inputs. Unaccounted carbon was ~13% and ~19% of the outputs for the PM and FM treatments, respectively, and ~24% of the inputs for the POLY-CAGE.

The carbon loads in the outlet water were ~14 – 32% of those in the inlet water for all treatments (Table 2). Thus, the culture process removed approximately 2,007, 1,643, 985, and 1,541 kg C-TC ha⁻¹ from the input water for the PM, FM, IMTA, and POLY-CAGE treatments, respectively, over 171 days. The organic carbon fraction of the outlet water showed no differences between treatments, whereas the

inorganic carbon was significantly higher in the IMTA treatment.

Feed carbon conversion for the prawn in the POLY-CAGE was significantly higher than that of the prawn in IMTA (Table 3). The animals in the POLY-CAGE converted a significantly higher proportion of feed carbon into harvested biomass (~49%) when compared to the PM and FM treatments but was similar to the IMTA treatment (~37%). The FM and IMTA treatments showed a similar proportion of carbon from all inputs converted into biomass (~17%), both of which were significantly higher than the PM and the POLY-CAGE.

Most correlation coefficients between tested variables showed significance except for the correlations between the total inorganic carbon concentration of the culture water and the dissolved oxygen, pH, and transparency (Table 4). The total carbon and total organic carbon concentrations of the water column were positively correlated with chlorophyll- α , total suspended solids, and the carbon content of the settleable solids, and were negatively correlated with transparency (Figs. 3 and 4). The total inorganic carbon showed moderate correlations only with the transparency and the total carbon content of the settleable solids (Fig. 5). No significant differences were shown between treatments for any of the sampling periods for the chlorophyll- α , TC, TOC, and TIC of the culture water (Fig. 6). The carbon content of the settleable solids showed no difference between treatments throughout the experiment, whereas the sedimentation rate of settleable solids showed significant differences at approximately 100 days of culture and thereafter, with the IMTA treatment having significantly higher rates of sedimentation when compared to the other treatments.

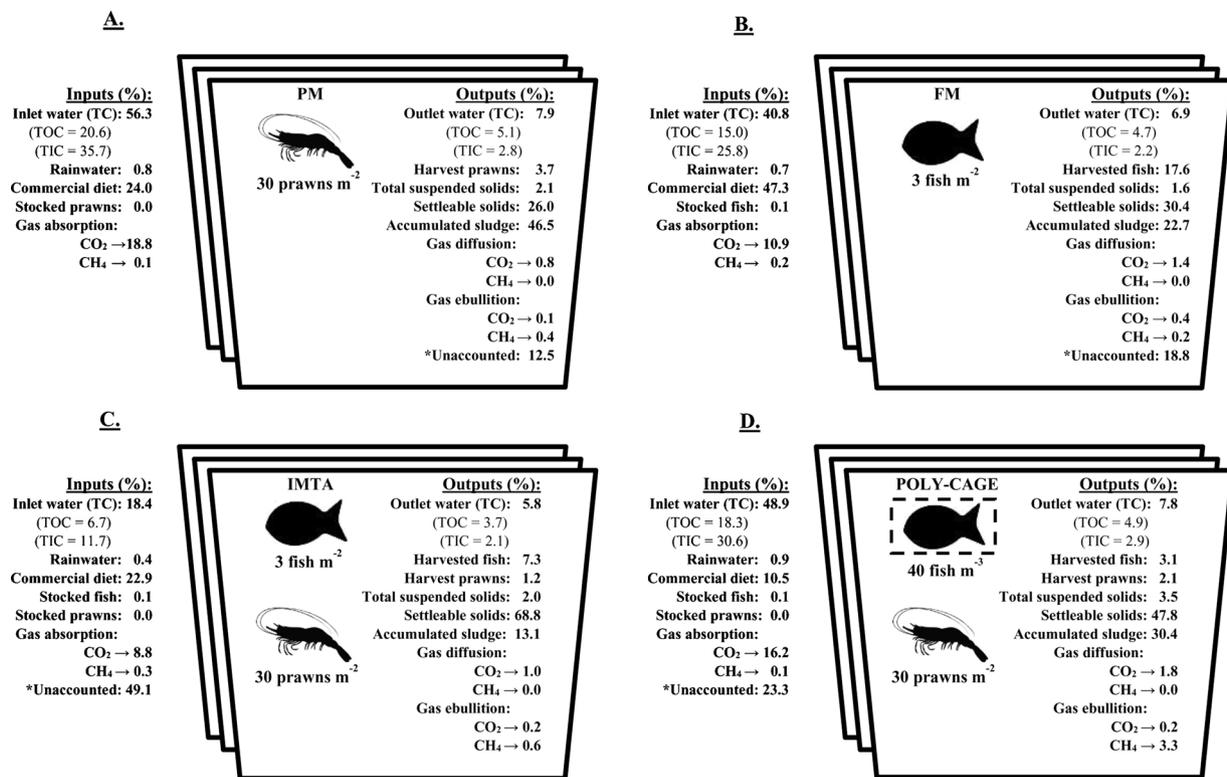


Fig. 1. Carbon budgets of the Amazon river prawn (*Macrobrachium amazonicum*) monoculture - PM (A), tambaqui fish (*Colossoma macropomum*) monoculture - FM (B), fish-prawn IMTA with the tambaqui reared free-swimming - IMTA (C), and fish-prawn IMTA with the tambaqui reared in cages - POLY-CAGE (D). Percentages (%) represent the fraction of each input and output of carbon over the total inputs and outputs of carbon, respectively. Total organic carbon (TOC) and total inorganic carbon (TIC) describe the composition of the total carbon (TC) in the inlet water and outlet water compartments and were not included when calculating the percent sum of inputs and outputs for each treatment. *Unaccounted represents unidentified processes that constitute sources of error in the carbon budget.

4. Discussion

The carbon budget showed that this element entered in the earthen pond systems mainly through the inlet water, commercial diet, and diffusion of atmospheric CO₂. The carbon then passed through the aquatic carbon cycle and accumulated as solid organic material in the bottom sediments. In general, the carbon contents in each compartment showed high variations between replicates of the same treatment. High variability of the data decreased the power of the ANOVA test (Sokal and Rohlf, 1995), inhibiting the detection of differences between the grow-out systems. This variability probably contributed to the large unaccounted portion in the mass balances as well. Nonetheless, the total carbon content of the inlet water (~1,438 – 2,335 kg C-TC ha⁻¹) was higher than that of the outlet water (~292 – 453 kg C-TC ha⁻¹), indicating that carbon from the source water was assimilated by the earthen pond systems. Furthermore, the absorption of atmospheric CO₂ (~529 – 782 kg C-CO₂ ha⁻¹) was much higher than the CO₂ and CH₄ emissions (~35 – 93 kg C-CO₂ ha⁻¹ and ~14 – 123 kg C-CH₄ ha⁻¹, respectively) in all treatments. Therefore, the grow-out production systems of these two species in earthen ponds can be considered as an important provider of ecosystem services for mitigating the eutrophication of receiving water bodies and sequestering atmospheric carbon dioxide.

The total carbon concentration of the culture water consists of all forms of dissolved and suspended organic and inorganic carbon, including live organisms. Chlorophyll- α ($r = 0.75$), total suspended solids ($r = 0.75$) and the organic carbon content of the settleable solids ($r = 0.79$) increased and transparency decreased ($r = -0.64$) according to the increasing total carbon concentration of the culture water. Except for the dissolved oxygen, all variables showed moderate to strong correlations with the total organic carbon concentration of the culture water ($r < -0.5$ or $r > 0.5$). The total inorganic carbon

content in the water column showed moderate correlations with the transparency ($r = -0.59$) and the organic carbon content of the settleable solids ($r = 0.62$). The strong positive correlations between chlorophyll- α and total suspended solids ($r = 0.79$) and with sedimentation ($r = 0.70$) indicate that most of these solids are composed of phytoplankton. The proliferation of the phytoplankton community occurs in the water column, but planktonic organisms have a short life span and settle on the pond bottoms as solid material after dying. Therefore, the results obtained from the correlation analyses suggest that primary production is a key process in pond aquaculture by absorbing CO₂ from atmospheric and autochthonous sources. Feed management has also been considered the major driver of biological processes in production ponds since commercial diets are a major allochthonous source of labile organic carbon, nitrogen, and phosphorus in fed aquaculture (Boyd et al., 2010; Flickinger et al., 2019, 2020). In the present study, the commercial diet showed a moderate correlation with the chlorophyll- α ($r = 0.65$). The inlet water was a major source of carbon as well, the majority of which was inorganic carbon. Dissolved inorganic carbon may be used for primary production and facilitate the uptake of other inorganic nutrients by phytoplankton (Wurts and Durbin, 1992; Kimpara et al., 2013).

Inlet water accounted for the highest proportion of input carbon for the *M. amazonicum* monoculture (~56%; ~499 mg m⁻² day⁻¹, ~866 mg m⁻² day⁻¹) and the second-highest input for the *C. macropomum* monoculture (~41%; ~427 mg m⁻² day⁻¹, ~731 mg m⁻² day⁻¹) and the IMTA (~18%; ~306 mg m⁻² day⁻¹, ~535 mg m⁻² day⁻¹). Reported inlet water contributions at the same facility were ~29 – 39% for the IMTA of the *M. amazonicum* and Nile tilapia *O. niloticus* (David, 2016). Other studies with fish polyculture and *M. rosenbergii* monoculture reported that inlet water contributed approximately 1% or less of all organic carbon inputs (Brown et al., 2012; Sahu et al., 2013; Adhikari et al., 2014). In the present study, the high carbon

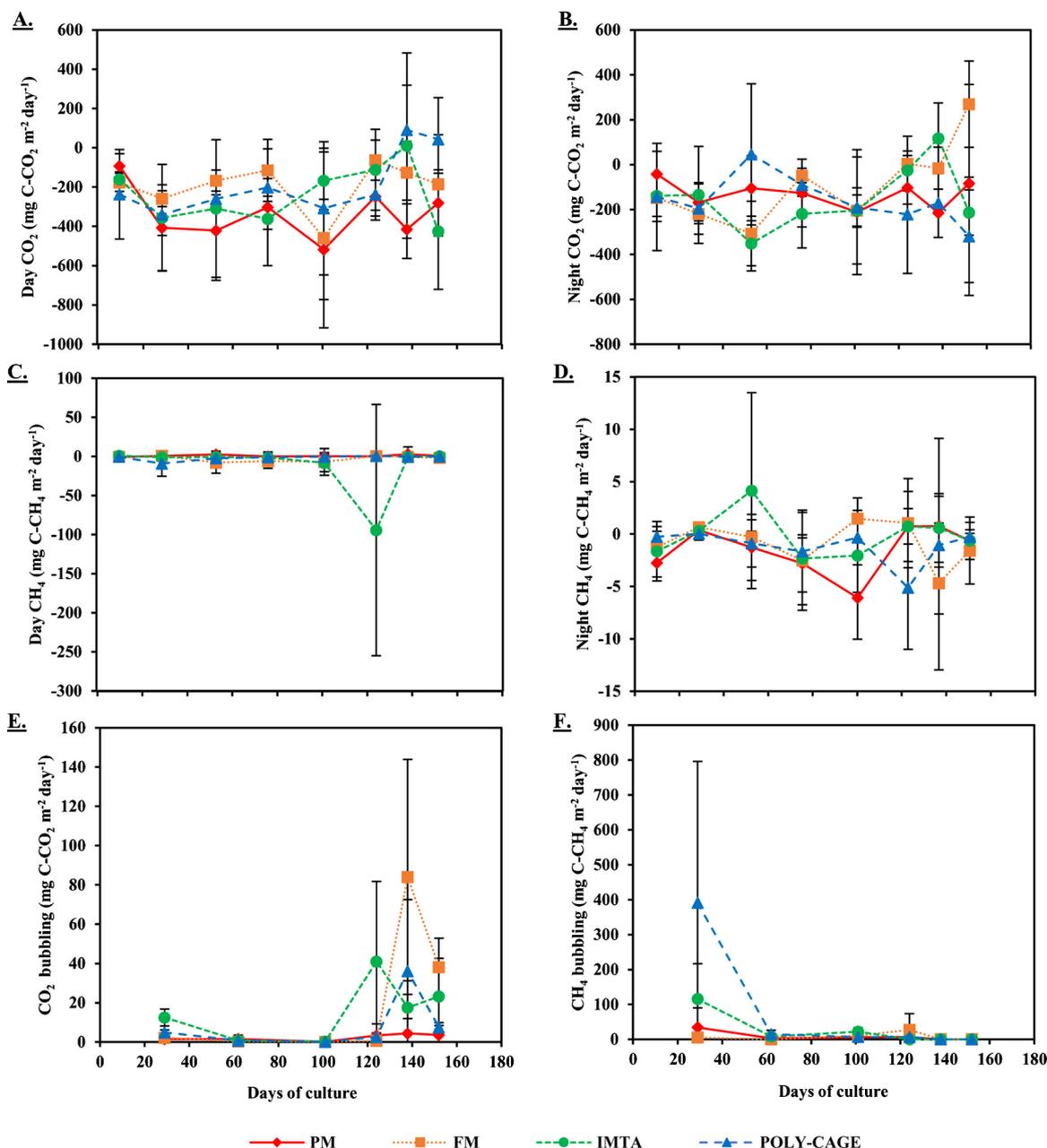


Fig. 2. Means (± SD) of daytime (A.) and nighttime (B.) fluxes (diffusion) of carbon – CO₂, and daytime (C.) and nighttime (D.) fluxes of carbon – CH₄ gases at the air-water interface; and the emissions of ebullitive CO₂ (E.) and CH₄ (F.) formed after biological processes during the grow-out of the Amazon river prawn (*Macrobrachium amazonicum*) and the tambaqui fish (*Colossoma macropomum*) in monoculture systems (Prawn Monoculture - PM and Fish Monoculture - FM), and in fish-prawn IMTA systems with the tambaqui reared as free-swimming (IMTA) and in cages (POLY-CAGE). Positive values represent carbon gas released to the atmosphere, and negative values represent carbon gas as an input (absorption).

contribution from the inlet water is due to the use of nutrient-rich water combined with the large volume used to replenish water lost through evaporation and seepage (~900 to 1,700 m³).

Feed usually accounts for a significant fraction of all nutrient inputs in aquaculture. Feed organic carbon in *M. rosenbergii* monocultures accounted for ~90 – 94% of all carbon inputs (Sahu et al., 2013; Adhikari et al., 2014) and in integrated fish grow-out systems feed was ~85 – 92% of carbon inputs (Gal et al., 2013). In the present study, feed organic carbon represented ~23 – 47% of all inputs for the two monocultures (~994 – 2298 kg C-TOC ha⁻¹) and the IMTA (~1799 ± 325 kg C-TOC ha⁻¹). The feed organic carbon for the IMTA of *M. amazonicum* and *O. niloticus* carried out at the same facility was ~58 – 63% of all inputs (David, 2016). The low proportion of feed organic carbon shown here when compared to prior studies is due to the

high carbon content of the source water and the inclusion of carbon dioxide as an input.

The feed carbon converted into harvested biomass for the *M. amazonicum* monoculture (~15%) was less than half of that observed for the *C. macropomum* monoculture (~37%) and IMTA (~37%), and nearly a third of that found for the polyculture with the fish reared in cages (~49%). The feed carbon conversions of the prawn in the latter treatment (~20%) was significantly higher than that of the prawn in IMTA (~5%) and was similar to that of the prawn in monoculture (~15%), despite the prawn in the polyculture receiving far less feed (~229 mg C-TOC m⁻² day⁻¹) than the other treatments (~581 – 1,344 mg C-TOC m⁻² day⁻¹). The feed carbon converted into *C. macropomum* biomass showed low variation between treatments, indicating that the carbon uptake by the *C. macropomum* is based on the

Table 3

Percent use efficiencies (mean \pm SD) of the carbon accumulated in the harvested biomass of the Amazon river prawn (*Macrobrachium amazonicum*) and tambaqui fish (*Colossoma macropomum*) grow-outs in monoculture systems (Prawn Monoculture - PM and Fish Monoculture - FM), and in fish-prawn IMTA systems with the tambaqui reared as free-swimming (IMTA) and in cages (POLY-CAGE).

	Treatments			
	PM	FM	IMTA	POLY-CAGE
Feed carbon conversion - FCC (%)				
Fish	-	36.8 \pm 3.5	31.9 \pm 2.5	29.2 \pm 7.4
Prawn	15.3 \pm 0.1 a	-	5.1 \pm 0.5 b	19.9 \pm 6.1 a
Total	15.3 \pm 0.1 c	36.8 \pm 3.5 b	37.0 \pm 2.2 ab	49.2 \pm 13.2 a
Total carbon use efficiency - CUE (%)				
Fish	-	17.4 \pm 2.5 a	14.4 \pm 0.7 a	4.1 \pm 1.6 b
Prawn	3.7 \pm 0.1	-	2.3 \pm 0.3	2.8 \pm 1.2
Total	3.7 \pm 0.1 b	17.4 \pm 2.5 a	16.7 \pm 0.6 a	6.9 \pm 2.8 b

Note. The feed carbon conversion was measured as the proportion (%) of the harvested biomass carbon (kg C-TOC ha⁻¹) over the carbon content of the commercial diet (kg C-TOC ha⁻¹), and the environmental carbon use efficiency (%) was considered as the harvested biomass carbon (kg C-TOC ha⁻¹) over the carbon from all inputs (kg C-TC ha⁻¹). Means followed by different letters in the same line indicate significant differences according to the Kruskal-Wallis test followed by the Wilcoxon rank-sum test.

allochthonous feed when considering that the fish in cages have limited access to natural feed sources and that the feed was administered in all treatments as a proportion of the fish biomass. Nevertheless, the total harvested biomass of both integrated grow-outs showed a more efficient conversion of the feed organic carbon (~37 – 49%) when compared to the monocultures (~15 – 37%). The prawn-tambaqui IMTA also increased the recovery of feed carbon when compared to the prawn-tilapia IMTA (~20 – 23%) (David, 2016), in which prawn were stocked at 21.5 animals m⁻². These results indicate that an increase in the stocking density of *M. amazonicum* in IMTA increases the conversion of feed nutrients, as suggested in previous studies (David et al. 2017a,

2017b; Flickinger et al., 2019, 2020).

Approximately 17% of the carbon from all inputs (CUE) was converted into harvested biomass in both fish monoculture and IMTA. The *C. macropomum* monoculture produced ~5.3 tonnes ha⁻¹ of harvested biomass, which is ~161.0 kg of organic carbon for each tonne of fish produced. Thus, this system recovered ~853.1 kg C-TOC ha⁻¹. By the same calculation, the IMTA recovered 133.7 kg of organic carbon for each tonne of harvested biomass of both animals, of which the fish accounted for ~133.0 kg C-TOC t⁻¹ of biomass. Previous studies have also reported low carbon use efficiencies of the animals for both monoculture and IMTA systems as well. In *M. rosenbergii* monoculture,

Table 4

Correlations between the total carbon [C-TC], total organic carbon [C-TOC] and total inorganic carbon [C-TIC] and other variables of the culture water; between chlorophyll- α and solid material, total suspended solids and the sedimentation of settleable solids, and between the commercial diet and the carbon content of the settleable solids during the grow-out of the Amazon river prawn (*Macrobrachium amazonicum*) and the tambaqui fish (*Colossoma macropomum*) in monoculture systems and their integrated grow-out in two IMTA systems.

Variable X	Variable Y	r	N	P
[C-TC] culture water (mg L ⁻¹)	Dissolved oxygen (mg L ⁻¹)	0.24	108	2.2E-2*
	pH	-0.45	108	2.1E-4*
	Chlorophyll- α (μ g L ⁻¹)	0.75	120	1.0E-8*
	Transparency (cm)	-0.64	108	2.4E-10*
	Commercial diet (kg day ⁻¹)	0.59	120	5.6E-10*
	Total suspended solids (mg L ⁻¹)	0.75	120	3.9E-15*
	[C-TOC] settleable solids (g kg ⁻¹)	0.79	84	1.1E-5*
	Sedimentation (kg SS ha ⁻¹ day ⁻¹)	0.58	84	6.2E-7*
	[C-TOC] culture water (mg L ⁻¹)	Dissolved oxygen (mg L ⁻¹)	0.33	108
pH		-0.55	108	6.7E-6*
Chlorophyll- α (μ g L ⁻¹)		0.77	120	3.9E-11*
Transparency (cm)		-0.77	108	2.2E-16*
Commercial diet (kg day ⁻¹)		0.63	120	9.5E-12*
Total suspended solids (mg L ⁻¹)		0.83	120	2.2E-16*
[C-TOC] settleable solids (g kg ⁻¹)		0.79	84	5.4E-6*
Sedimentation (kg SS ha ⁻¹ day ⁻¹)		0.65	84	2.2E-7*
[C-TIC] culture water (mg L ⁻¹)		Dissolved oxygen (mg L ⁻¹)	0.04	108
	pH	-0.15	108	0.2
	Chlorophyll- α (μ g L ⁻¹)	0.44	120	9.3E-3*
	Transparency (cm)	-0.59	108	0.1
	Commercial diet (kg day ⁻¹)	0.37	120	1.2E-3*
	Total suspended solids (mg L ⁻¹)	0.42	120	5.3E-3*
	[C-TOC] settleable solids (g kg ⁻¹)	0.62	84	8.5E-3*
	Sedimentation (kg SS ha ⁻¹ day ⁻¹)	0.33	84	3.7E-2*
	Chlorophyll- α (μ g L ⁻¹)	Total suspended solids (mg L ⁻¹)	0.79	120
[C-TOC] settleable solids (g kg ⁻¹)		0.49	84	3.7E-2*
Sedimentation (kg SS ha ⁻¹ day ⁻¹)		0.70	84	1.9E-6*
Total suspended solids (mg L ⁻¹)		0.78	84	2.7E-9*
Commercial diet (kg day ⁻¹)	Chlorophyll- α (μ g L ⁻¹)	0.65	120	1.9E-7*
	[C-TOC] settleable solids (g kg ⁻¹)	0.45	84	9.6E-4*

Note. r = Pearson correlation coefficient. N = number of samples from all treatments. *The correlation coefficient between the variables was considered significant according to the t-test (P < 0.05)

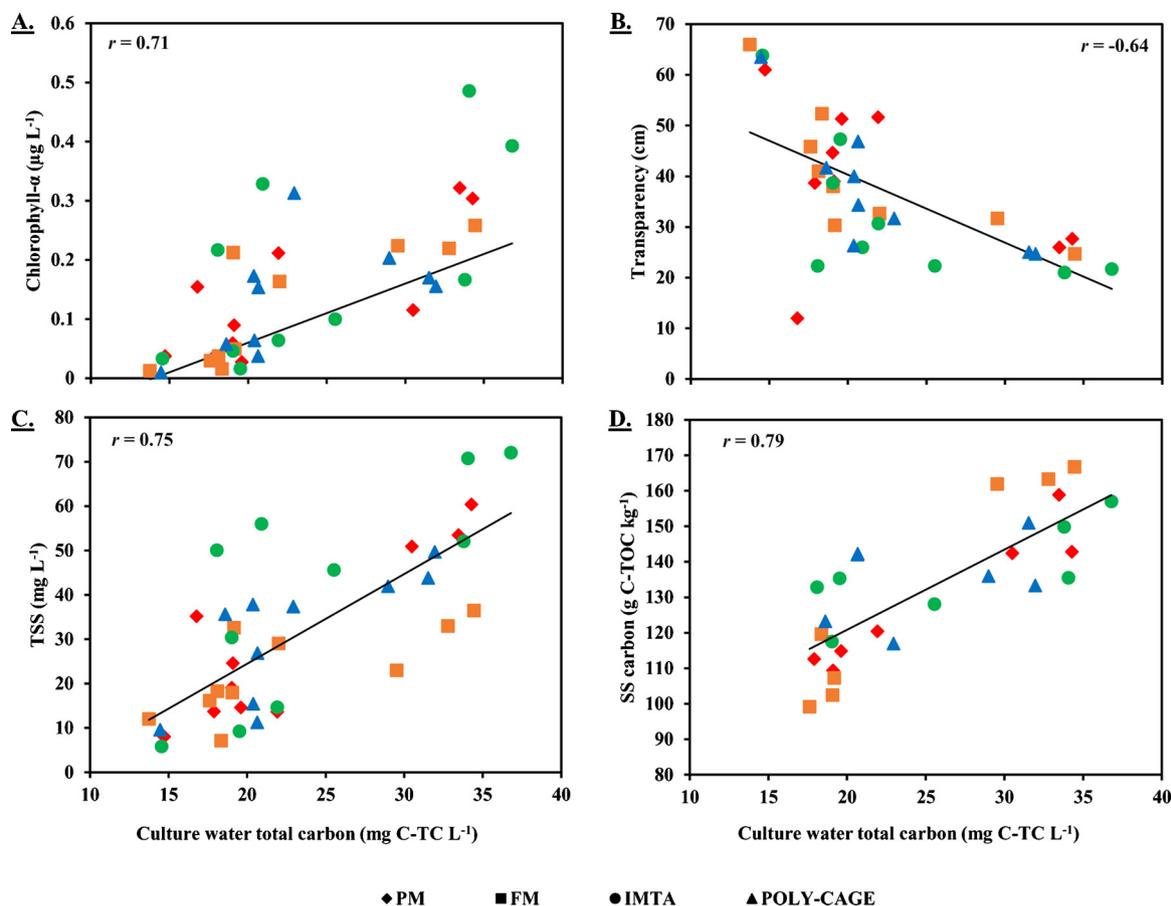


Fig. 3. Correlation between the culture water total carbon concentrations and the chlorophyll- α (A.), transparency (B.), total suspended solids (TSS) (C.) and carbon concentration of the settleable solids (SS) (D.) during the grow-out of the Amazon river prawn (*Macrobrachium amazonicum*) and the tambaqui fish (*Colossoma macropomum*) in monoculture systems (Prawn Monoculture - PM and Fish Monoculture - FM), and in fish-prawn IMTA systems with the tambaqui reared as free-swimming (IMTA) and in cages (POLY-CAGE).

assimilation was $\sim 15 - 16\%$ of all inputs based on the concentrations of the total organic carbon (Sahu et al., 2013; Adhikari et al., 2014). In the IMTA of *M. amazonicum* and *O. niloticus*, the harvested biomass assimilated $\sim 13\%$ of carbon from all inputs (David, 2016). In the present study, the low proportion of carbon accumulated as harvested biomass suggests that these species farmed in IMTA are still inefficient in the utilization of carbon available inside the ponds. Therefore, a higher density of prawns or the addition of an iliophagus (mud-feeder) species should be investigated to improve the uptake of carbon.

The *M. amazonicum* monoculture of the present study converted nearly 4% of carbon from all inputs into biomass, whereas the unfed prawn in the integrated grow-outs recovered $\sim 2 - 3\%$ of the carbon from all inputs. The conversion of carbon from all inputs into prawn biomass was higher ($\sim 15 - 16\%$) for *M. rosenbergii* in fed monocultures (Sahu et al., 2013; Adhikari et al., 2014). This is perhaps due to the inclusion of inorganic carbon and atmospheric CO_2 as inputs in the present study, which increased the sum of all carbon inputs and decreased the proportion of carbon retained in the prawn biomass when compared to the previous studies. Nonetheless, the carbon removed by the harvested prawn biomass in monoculture was $152.0 \pm 30.5 \text{ kg C-TOC ha}^{-1}$ and in the integrated cultures was $\sim 77.3 - 91.6 \text{ kg C-TOC ha}^{-1}$, much lower than the $\sim 80 - 294 \text{ kg C-TOC ha}^{-1}$ recorded for the monocultures of the *M. rosenbergii* (Sahu et al., 2013; Adhikari et al., 2014). The low organic carbon retentions of the *M. amazonicum* may be due to its small size and its lack of specific feed and feed preferences (Moraes-Valenti and Valenti, 2010). In contrast to the prawn in monoculture, those in the integrated cultures fed on bottom fauna, fish feces, and feed wastes, making the carbon retained by the prawn in

IMTA a 100% gain since no feed was supplied to the prawn during the entire culture cycle. The rearing of the *M. amazonicum* as an unfed secondary species is ideal for freshwater IMTA due to its omnivorous and detritivorous feeding habits, its restriction to the epibenthic zone, its preference for sediment-rich waters, and that most of the nutrients and organic residues from all inputs accumulate in the upper layers of bottom sediments (Moraes-Valenti and Valenti, 2010; Marques and Moraes-Valenti, 2012).

In the present study, carbon contents of the total suspended solids, the settleable solids, and the accumulated sludge accounted for $\sim 55 - 84\%$ of the outputs ($\sim 2,655 - 6,573 \text{ kg C-TOC ha}^{-1}$). The total suspended solids were only $\sim 2 - 4\%$ of all carbon outputs ($\sim 77 - 155 \text{ kg C-TOC ha}^{-1}$). This small amount of carbon contained in suspended solids is probably comprised of photosynthetic phytoplankton, as indicated by its high correlation with the chlorophyll- α ($r = 0.79$). The consistent absorption of atmospheric CO_2 and the steady increase in the settleable solids throughout the experimental period suggest high photosynthetic activity and phytoplankton turnover. In this manner, organic carbon is rapidly produced in the water column and settles to the bottom when the plankton dies.

The carbon content of the settleable solids and accumulated sludge accounted for $\sim 53 - 82\%$ of outputs in all treatments ($\sim 2,578 - 6,418 \text{ kg C-TOC ha}^{-1}$). Bottom sediments in *M. rosenbergii* monocultures, fish polyculture and the IMTA of *M. amazonicum* and *O. niloticus* were reported to have high retentions of organic carbon as well, ranging from $\sim 42 - 81\%$ (Nhan et al., 2008; Sahu et al., 2013; Adhikari et al., 2014; David, 2016). The bottom sediments have been suggested to accumulate $\sim 25\%$ of organic carbon from commercial feed inputs

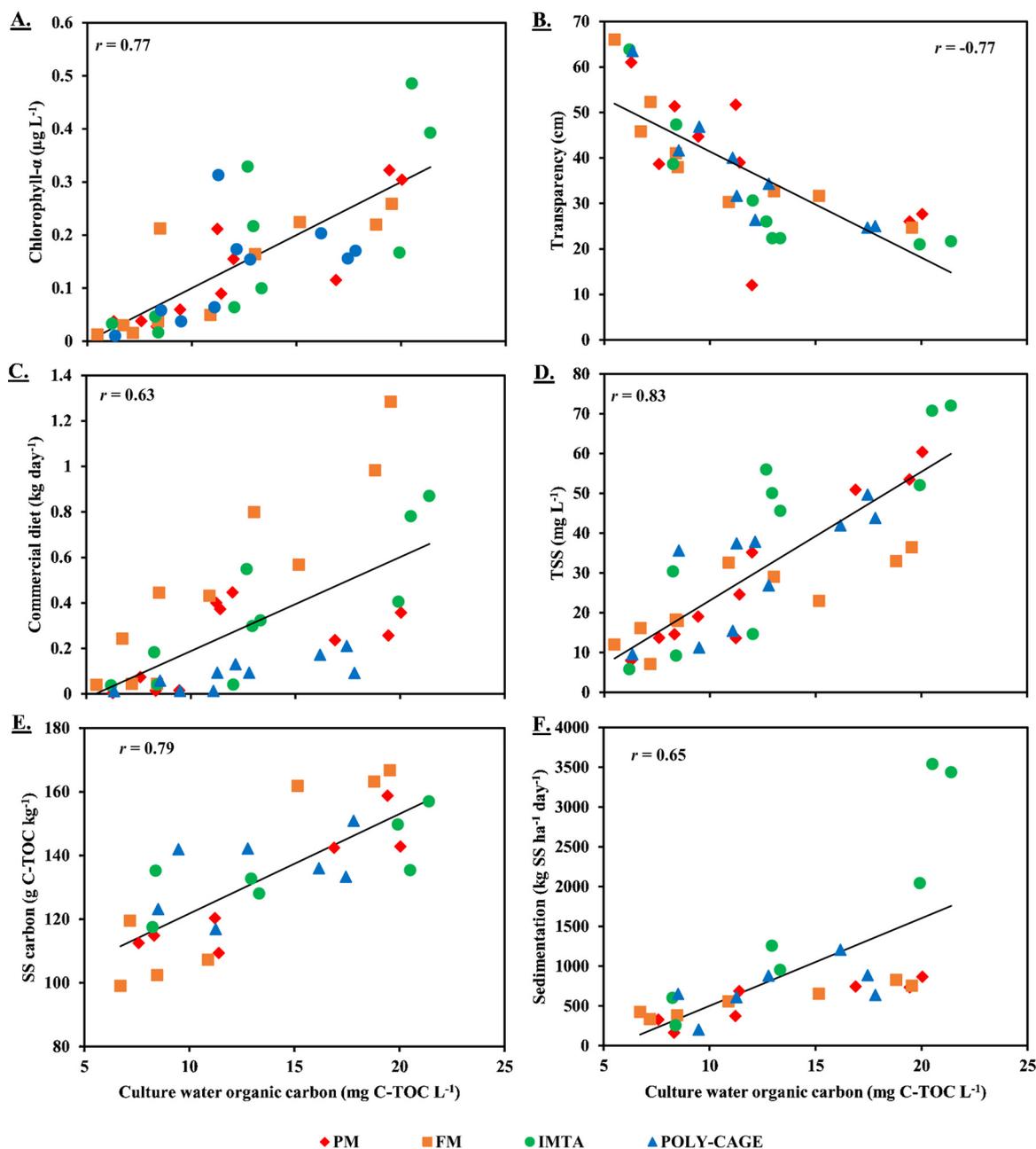


Fig. 4. Correlation between the in-pond total organic carbon concentrations and the chlorophyll- α (A.), transparency (B.), diet (C.), total suspended solids (TSS) (D.), total carbon concentration of the settleable solids (SS) (E.) and the daily sedimentation of settleable solids (F.) during the grow-out of the Amazon river prawn (*Macrobrachium amazonicum*) and the tambaqui fish (*Colossoma macropomum*) in monoculture systems (Prawn Monoculture - PM and Fish Monoculture - FM), and in fish-prawn IMTA systems with the tambaqui reared as free-swimming (IMTA) and in cages (POLY-CAGE).

while a small portion is retained in the animal biomass, and the rest is mineralized as CO_2 (Avnimelech and Lacher, 1979). In the present study, the carbon accumulated in the sludge was approximately four to six times that provided by commercial feed for the *M. amazonicum* monoculture and the polyculture with the fish reared in cages, and in the *C. macropomum* monoculture and IMTA, the accumulated sludge carbon was $\sim 48\%$ and $\sim 57\%$ of the feed carbon, respectively. Perhaps, the high organic carbon content in the sludge observed in the present study is partially due to the high clay content of the native soils, which have a high carrying capacity for nutrients (Amorim and Batalha, 2006; Silveira et al., 2016; Levinski-Huf and Klein, 2018). The carbon loads of the accumulated sludge ranged between $\sim 1,028$ and $1,139 \text{ kg C-TOC ha}^{-1}$ for the *C. macropomum* monoculture and the two integrated cultures whereas the *M. amazonicum* monoculture varied higher at

$\sim 1,931 \text{ kg C-TOC ha}^{-1}$, which is expected with the use of sinking feed (Avnimelech and Ritvo, 2003).

In addition to the feed, atmospheric CO_2 may have contributed to the accumulated organic carbon in the settleable solids and sludge. The inorganic carbon load of the outlet water ($\sim 106 - 164 \text{ kg C-TIC ha}^{-1}$) and the CO_2 emissions represented less than 5% of all carbon outputs in all treatments. The low CO_2 emission and inorganic carbon content of the water column and the consistent input of atmospheric CO_2 ($\sim 310 - 457 \text{ kg C-CO}_2 \text{ ha}^{-1}$) suggest that this greenhouse gas was rapidly immobilized by photosynthetic organisms. In addition, the high input of atmospheric CO_2 and the high accumulation of carbon in the settleable solids ($\sim 631 - 3,152 \text{ mg C-TOC m}^{-2} \text{ day}^{-1}$) suggest high sequestration and rapid conversion of carbon gas by phytoplankton (Moriarty, 1997; Boyd et al. 2010).

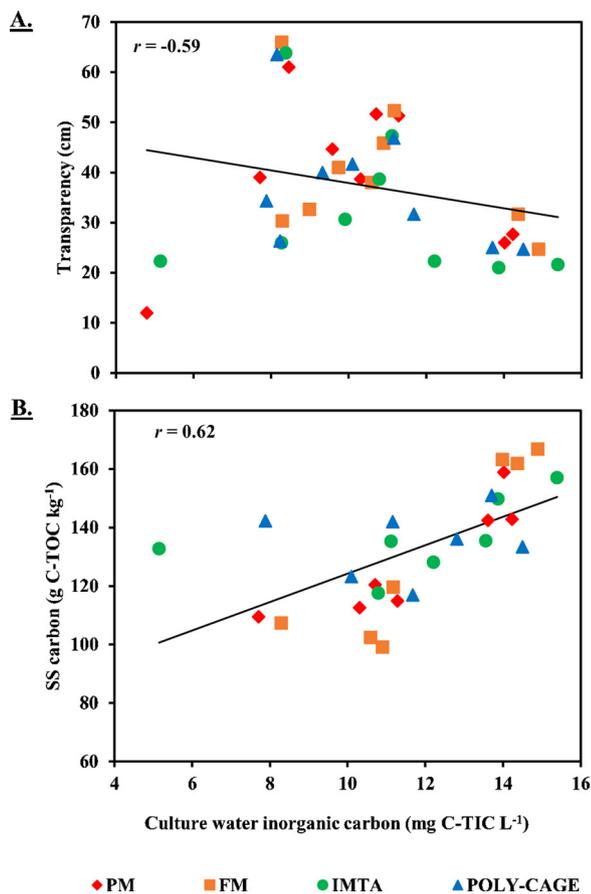


Fig. 5. Correlation between the culture water total inorganic carbon concentrations and the transparency (A) and the total carbon of the settleable solids (SS) (B.) during the grow-out of the Amazon river prawn (*Macrobrachium amazonicum*) and the tambaquifish (*Colossoma macropomum*) in monoculture systems (Prawn Monoculture - PM and Fish Monoculture - FM), and in fish-prawn IMTA systems with the tambaqui reared as free-swimming (IMTA) and in cages (POLY-CAGE).

A major issue for the expansion of aquaculture activities is the emission of greenhouse gases toward the atmosphere (Boyd et al., 2010; Clark and Tilman, 2017). The present study showed that the absorption of atmospheric CO₂ (~538 – 787 kg C-CO₂ ha⁻¹) was approximately six to 23 times that released to the atmosphere (~35 – 93 kg C-CO₂ ha⁻¹). Furthermore, atmospheric CO₂ was absorbed throughout the experimental period. These results corroborate previous hypotheses that freshwater earthen ponds used for aquaculture have a high capacity to absorb atmospheric CO₂ and accumulate carbon as solid organic matter in the bottom sediments with little adverse impacts on the water quality (Boyd et al., 2010).

Carbon dioxide can be released toward the atmosphere via mineralization of organic carbon, carbonate dissolution, and methanogenesis (Gruca-Rokosz and Koszelnik, 2018). Outputs of carbon gases were shown to represent ~2 – 4% of all outputs for the IMTA of *M. amazonicum* and *O. niloticus* in earthen ponds (David, 2016). In the present study, CO₂ diffusion represented ~1 – 2% of all outputs (~32 – 74 kg C-CO₂ ha⁻¹), with no differences shown between treatments. Ebullitive CO₂ became more frequent toward the end of the experimental period but was nearly negligible, representing ~0.2 – 0.4% of all outputs (~2 – 11 mg C-CO₂ m⁻² day⁻¹). The occurrence of CO₂ bubbles was perhaps due to the increasing accumulation of organic material as settleable solids over time. Mineralized CO₂ in the bottom sediments can diffuse upward toward the water column and percolate downward with the seepage water. Methanogenesis occurs in anoxic layers of the bottom sediments and uses CO₂ as the terminal electron acceptor

(Gruca-Rokosz et al., 2011). Methane (CH₄) was less than 1% of outputs for the two monocultures and IMTA and was ~3% of all outputs for the polyculture with the fish reared in cages. Most of the CH₄ emission was observed for the first two samples, and traces were detected in gas bubbles throughout the rest of the experiment (~7 – 72 mg C-CH₄ m⁻² day⁻¹). The composition of the ebullitive CO₂ suggests that carbon was mineralized from the bottom sediments and was used for methanogenesis (Gruca-Rokosz and Koszelnik, 2018), of which the ebullitive CH₄ was oxidized to CO₂ upon being released toward the oxygenated water column.

All treatments of the present study showed a steady increase of the settleable solids over time and, consequently, an increase in the carbon accumulated as settleable solids. All treatments were comparatively similar for the carbon concentrations of the settleable solids (~130 – 135 g C-TOC kg⁻¹ SS) and of the accumulated sludge (~43 – 53 g C-TOC kg⁻¹ AS), indicating that the bottom sediments may have reached a steady-state (Avnimelech, 1984; Avnimelech and Wodka, 1988). In other words, the accumulation of autochthonous and allochthonous organic carbon were equal to the mineralization of carbonaceous material. Intrinsic CO₂ is then cycled within the earthen pond system between being fixated by photosynthetic phytoplankton and returning to the bottom sediments as organic matter from phytoplankton turnover. The intrinsic CO₂ produced from aerobic decomposition in a steady-state system is also equal to the photoautotrophic production of oxygen, which is cycled internally as well (Moriarty, 1997). Furthermore, these earthen pond systems reduced the nitrogen load from the inlet water through retention in the bottom sediments followed by denitrification and the release of N₂ bubbles to the atmosphere (Flickinger et al., 2019). Therefore, the semi-intensive monocultures and IMTA of the *M. amazonicum* and *C. macropomum* can provide valuable ecosystem services, retaining carbon contained in the inlet water and absorbing atmospheric carbon dioxide. Thus, these aquaculture systems may be eligible for nitrogen and carbon trading credits.

The IMTA system accumulates more carbon in the pond bottom than the other systems. This finding suggests a higher autochthonous accumulation of organic material from phytoplankton growth and turnover (Avnimelech et al., 2001; Hargreaves, 2006). Perhaps, the higher bioturbation produced by both the prawn and fish together expose more buried organic carbon sources to aerobic decomposition and liberation of nutrients to the water column, increasing the photosynthesis and fixation of carbon (Green, 1992; Joyni et al., 2011). Another hypothesis to explain this high value of sediments in the IMTA system would be a failure to measure the inputs of carbon in the ponds caused by methodological limitations or by neglecting any important compartment when designing the budget. The inorganic carbon content of the outlet water was significantly higher for the IMTA system (164.2 ± 9.4 kg C-TIC ha⁻¹) as well, which is expected since an increase in organic material leads to higher rates of aerobic decomposition and the subsequent mineralization of CO₂ (Moriarty, 1997; Gruca-Rokosz et al., 2011).

The unidentified compartments accounted for ~13 – 19% of outputs for the two monocultures and ~23 – 49% of inputs for the two integrated cultures. David (2016) showed that the unidentified portions accounted for ~1 – 27% of the outputs in the IMTA of *O. niloticus* and *M. amazonicum* carried out at the same facility. Studies with freshwater prawn indicate percentages of unquantified carbon ranging from ~17 – 19% of the outputs (Sahu et al., 2013; Adhikari et al., 2014). Unaccounted portions are difficult to compare between studies due to the different species used, soil and water qualities, applied analytical techniques, and the general lack of data. Imprecise measures related to the bottom soil and gas exchange might have affected the accuracy of the carbon mass balances in the present study. The unquantified outputs of carbon in the two monocultures may be related to the microbial decomposition of settled organic carbon, of which the mineralized CO₂ can percolate through the bottom sediments with the seepage water (Wurts and Durborow, 1992). The unquantified inputs of carbon in the two integrated cultures may be related to animal bioturbation of the

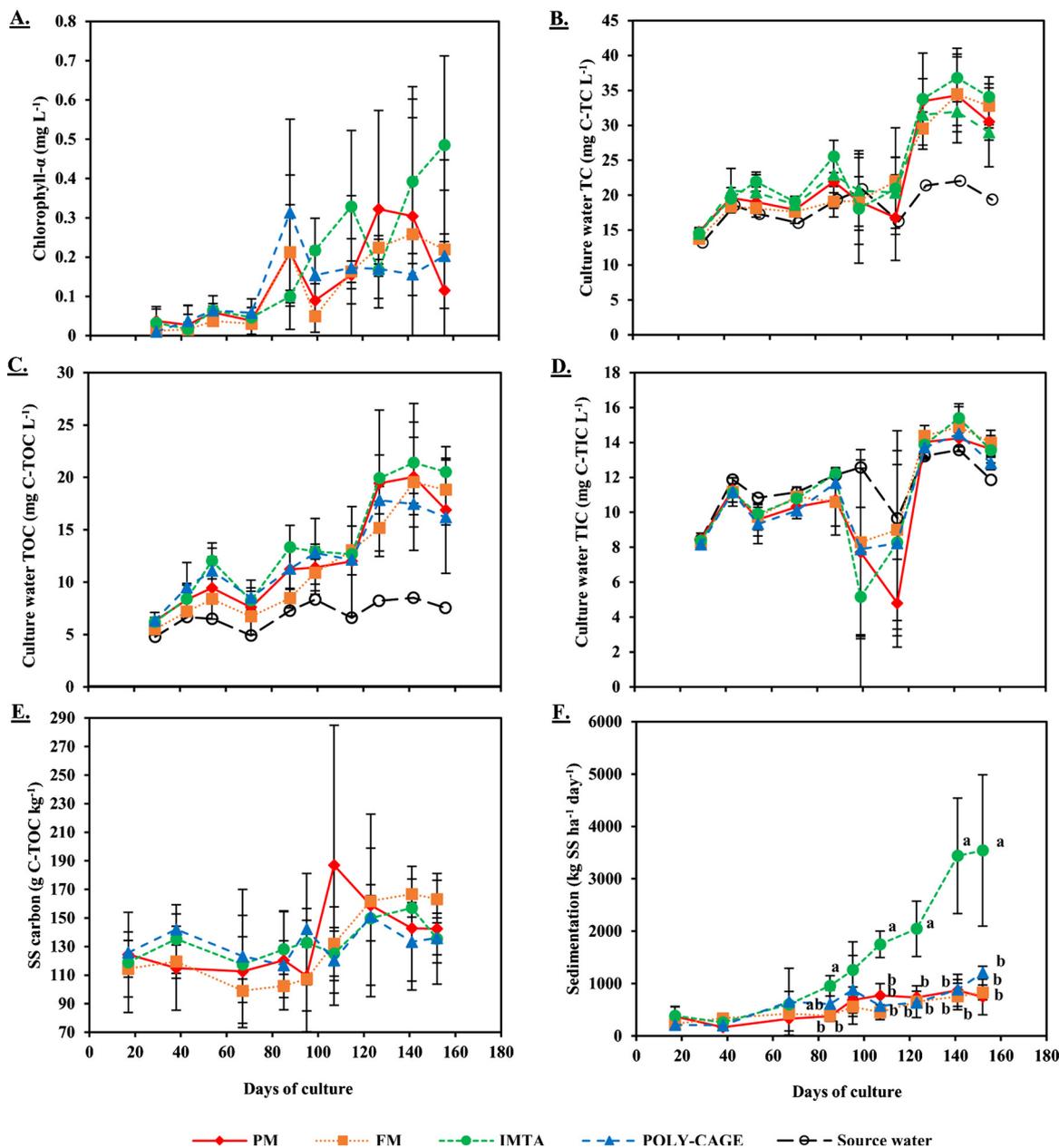


Fig. 6. Means (\pm SD) for the culture water concentrations of chlorophyll- α (A.), total carbon (TC) (B.), total organic carbon (TOC) (C.), and total inorganic carbon (TIC) (D.), and total carbon of the settleable solids (SS) (E.) and the daily sedimentation of settleable solids (F.) during the grow-out of the Amazon river prawn (*Macrobrachium amazonicum*) and the tambaqui fish (*Colossoma macropomum*) in monoculture systems (Prawn Monoculture - PM and Fish Monoculture - FM), and in fish-prawn IMTA systems with the tambaqui reared as free-swimming (IMTA) and in cages (POLY-CAGE). Source water organic, inorganic, and total carbon concentrations were measured but not included in the statistical analyses with the treatments. Different letters indicate significant differences between treatments according to the Tukey test ($P < 0.05$), and * indicates that differences were determined using the Kruskal-Wallis test followed by the Wilcoxon rank-sum test.

bottom sediments, of which the bottom soil was not considered as an input. The foraging behavior of the prawns in the integrated cultures may have exposed buried organic carbon to aerobic processes (Martin et al., 1998; Boyd et al., 2010; Kimpara et al., 2011). Pond bottom soil is difficult to measure as a carbon input when considering that the effects of bioturbation on the movement of nutrients are little known and that the release of nutrients depends on soil and water conditions (Joyni et al., 2011). The present study was the second carbon mass balance analysis in pond aquaculture to include the direct measures of carbon gas exchange. However, gas analyses showed high variation, and some of the samples were unviable. Thus, further research is necessary to understand how bottom soils act as a nutrient source, and gas analyses should include a higher sample size for a more accurate estimation of

gas exchange.

In conclusion, the present study contributes to filling data gaps in the understanding of the carbon cycle in freshwater aquaculture carried out in earthen ponds. Atmospheric CO₂ represented a high proportion of the carbon inputs, and its absorption by the earthen ponds was consistent throughout the experimental period, whereas carbon gas emissions were negligible. Furthermore, these earthen pond grow-out systems showed resilience when considering that the high carbon inputs of the supply water, feed, and atmospheric gases were disseminated among all compartments rather than accumulating in the water column. The IMTA system showed the greatest potential for intensification since water quality variables were maintained within acceptable ranges despite the high accumulation of organic carbon in the

settleable solids. The IMTA may benefit the earthen pond system by converting buried organic carbon to settleable organic material, which can be used after harvesting for plant culture inside ponds or removed and used for other agricultural activities. An increased prawn density, manipulation of the C:N ratio in feed, or the addition of a mud-feeder species to the culture may enhance the incorporation of settled autochthonous and allochthonous carbon sources into harvested biomass, improving the efficiency of the systems. Further research should be performed to understand the bioavailability of organic carbon accumulated in the settleable solids, sludge, and the water column, including the effects that bioturbation from the farmed species has on biological processes in the bottom sediments.

5. Credit Author Statement

Dallas L. Flickinger: Conducted the experiment, Data acquirement and curation, Data Analyses and Interpretation, Written original draft, Written-review and editing.

Gelcirene A. Costa: Conducted the experiment, data acquirement and curation, Written original draft.

Daniela P. Dantas: Conducted the experiment, data acquirement and curation.

Danielo C. Proença: Data Analyses and Interpretation.

Fernanda S. David: Data Analyses and Interpretation.

Robert M. Durborow: Investigation, Interpretation of data.

Patricia Moraes-Valenti: Conceptualization, Supervision, Project administration

Wagner C. Valenti: Conceptualization, Formal Analyses, Fund acquisition, Supervision, Interpretation of data, Written-review and editing.

6. Declarations of interest

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.aqrep.2020.100340>.

References

- Adhikari, S., Sahu, B.C., Mahapatra, A.S., Dey, L., 2014. Nutrient budgets and effluent characteristics in Giant freshwater prawn (*Macrobrachium rosenbergii*) culture ponds. *Bulletin of Environmental Contamination and Toxicology* 92, 509–513. <https://doi.org/10.1007/s00128-014-1227-4>.
- Ainsworth, R.F., Cowx, I.G., 2018. Validation of FAO inland fisheries catch statistics and replacement of fish with equivalent protein sources. Unpublished report to FAO.
- Amorim, P.K., Batalha, M.A., 2006. Soil characteristics of a hyperseasonal cerrado compared to a seasonal cerrado and a floodplain grassland: implications for plant community structure. *Brazilian Journal of Biology* 66, 661–670. <https://doi.org/10.1590/S1519-69842006000400010>.
- AOAC (Association of Official Analytical Chemists), 1995. *Official methods of analysis*. AOAC International, Washington, District of Columbia, USA.
- APHA (American Public Health Association), 2005. *Standard methods for the examination of water and waste water*. APHA, Washington, District of Columbia, USA.
- Avnimelech, Y., 1984. Reactions in fish pond sediments as inferred from sediment cores data. In: In: Rosenthal, H., Sarig, S. (Eds.), *Research on Aquaculture*. European Mariculture Society Vol. 8. Special Publication, Bredene, Belgium, pp. 41–54.
- Avnimelech, Y., Lacher, M., 1979. A tentative nutrient budget for intensive fish ponds. *Bamidgeh. Israeli Journal of Aquaculture* 31, 3–8.
- Avnimelech, Y., Ritvo, G., 2003. Shrimp and fish pond soils: processes and management. *Aquaculture* 220, 549–567. [https://doi.org/10.1016/S0044-8486\(02\)00641-5](https://doi.org/10.1016/S0044-8486(02)00641-5).
- Avnimelech, Y., Ritvo, G., Meijer, L.E., Kochba, M., 2001. Water content, organic carbon and dry bulk density in flooded sediments. *Aquacultural Engineering* 25, 25–33. [https://doi.org/10.1016/S0144-8609\(01\)00068-1](https://doi.org/10.1016/S0144-8609(01)00068-1).
- Avnimelech, Y., Wodka, M., 1988. Accumulation of nutrients in the sediments of MaalehHakishon reclaimed effluents reservoir. *Water Research* 22, 1437–1442. [https://doi.org/10.1016/0043-1354\(88\)90101-7](https://doi.org/10.1016/0043-1354(88)90101-7).
- Boyd, C.E., 1985. Chemical budgets for channel catfish ponds. *Transactions of the American Fisheries Society* 114, 291–298. [https://doi.org/10.1577/1548-8659\(1985\)114<291:CBFCCP>2.0.CO;2](https://doi.org/10.1577/1548-8659(1985)114<291:CBFCCP>2.0.CO;2).
- Boyd, C.E., 1995. *Bottom Soils, Sediment, and Pond Aquaculture*. Chapman & Hall, New York, USA, pp. 348.
- Boyd, C.E., 2015. *Water quality: an introduction*. Springer.
- Boyd, C.E., Teichert-Coddington, D., 1995. Dry matter, ash, and elemental composition of pond-cultured *Penaeus vannamei* and *Penaeus stylirostris*. *Journal of the World Aquaculture Society* 26, 88–92. <https://doi.org/10.1111/j.1749-7345.1995.tb00214.x>.
- Boyd, C.E., Wood, C.W., Chaney, P.L., Queiroz, J.F., 2010. Role of aquaculture pond sediments in sequestration of annual global carbon emissions. *Environmental Pollution* 158, 2537–2540. <https://doi.org/10.1016/j.envpol.2010.04.025>.
- Briggs, M.R.P., Funge-Smith, S.J., 1994. A nutrient budget of some intensive marine shrimp ponds in Thailand. *Aquaculture Research* 25, 789–811. <https://doi.org/10.1111/j.1365-2109.1994.tb00744.x>.
- Brown, T.W., Boyd, C.E., Chappell, J.A., 2012. Approximate water and chemical budgets for an experimental, in-pond raceway system. *Journal of the World Aquaculture Society* 43, 526–537. <https://doi.org/10.1111/j.1749-7345.2012.00576.x>.
- Casillas-Hernández, R., Magallón-Barajas, F., Portillo-Cark, G., Páez-Osuna, F., 2006. Nutrient mass balances in semi-intensive shrimp ponds from Sonora, Mexico using two feeding strategies: trays and mechanical dispersal. *Aquaculture* 258, 289–298. <https://doi.org/10.1016/j.aquaculture.2006.03.027>.
- Chopin, T., Cooper, J.A., Reid, G., Cross, S., Moore, C., 2012. Open-water integrated multi-trophic aquaculture: environmental biomitigation and economic diversification of fed aquaculture by extractive aquaculture. *Reviews in Aquaculture* 4, 209–220. <https://doi.org/10.1111/j.1753-5131.2012.01074.x>.
- Clark, M., Tilman, D., 2017. Comparative analysis of environmental impacts of agricultural production systems, agricultural input efficiency, and food choice. *Environmental Research Letters* 12, e064016. <https://doi.org/10.1088/1748-9326/aa6cd5>.
- Cunha, V.V., Santos Junior, A., 2011. Crescimento de juvenis de tambaqui, *Colossoma macropomum* (CUVIER, 1818), em tanques-rede com diferentes densidades populacionais em Ji-Paraná. RO. Amazônia: Ciência & Desenvolvimento 6, 185–193. <https://doi.org/10.1590/1809-4392201402205>.
- Daniels, H.V., Boyd, C.E., 1989. Chemical Budgets for Polyethylene-lined, Brackishwater Ponds. *Journal of the World Aquaculture Society* 20, 53–59. <https://doi.org/10.1111/j.1749-7345.1989.tb00524.x>.
- Dantas, D.P., Flickinger, D.L., Costa, G.A., Batlouni, S.R., Moraes-Valenti, P., Valenti, W.C., 2020. Technical feasibility of integrating Amazon river prawn culture during the first phase of tambaqui grow-out in stagnant ponds, using nutrient-rich water. *Aquaculture* 516 (1 February), 734611. <https://doi.org/10.1016/j.aquaculture.2019.734611>.
- David, F.S., 2016. *Balço de nitrogênio, fósforo e carbono em sistema multiespacial e multitrófico de tilápia-do-nilo e camarão-da-amazônia*. Doctorate thesis. Aquaculture Center of São Paulo State University, Jaboticabal, pp. 93.
- David, F.S., Proença, D.C., Valenti, W.C., 2017a. Nitrogen budget in integrated aquaculture systems with Nile tilapia and Amazon river prawn. *Aquaculture International* 25, 1733–1746. <https://doi.org/10.1007/s10499-017-0145-y>.
- David, F.S., Proença, D.C., Valenti, W.C., 2017b. Phosphorus Budget in Integrated Multi-Trophic Aquaculture Systems with Nile Tilapia (*Oreochromis niloticus*) and Amazon river prawn (*Macrobrachium amazonicum*). *Journal of the World Aquaculture Society* 48, 402–414. <https://doi.org/10.1111/jwas.12404>.
- Ferrari, V.A., Lucas, A.F.B., Gaspar, L.A., 1991. Desempenho do tambaqui *Colossoma macropomum* Cuvier, 1818, em monocultura experimental sob condições de viveiro-estufa e viveiro convencional (2ª fase) no sudeste do Brasil. *Boletim Técnico do CEPTA* 4, 23–37.
- Flickinger, D.L., Costa, G.A., Dantas, D.P., Moraes-Valenti, P., Valenti, W., 2019. The budget nitrogen in the grow-out of the Amazon river prawn (*Macrobrachium amazonicum* Heller) and tambaqui (*Colossoma macropomum* Cuvier) farmed in monoculture and integrated multitrophic aquaculture systems. *Aquaculture Research* 50, 3444–3461. <https://doi.org/10.1111/are.14304>.
- Flickinger, D.L., Dantas, D.P., Proença, D.C., David, F.S., Valenti, W.C., 2020. Phosphorus in the culture of the Amazon river prawn (*Macrobrachium amazonicum*) and tambaqui (*Colossoma macropomum*) farmed in monoculture and in integrated multi-trophic systems. *Journal of the World Aquaculture Society*. In Press. <https://doi.org/10.1111/jwas.12655>.
- Gal, D., Pekar, F., Kosaros, T., Kerepeczki, E., 2013. Potential of nutrient reutilization in combined intensive-extensive pond systems. *Aquaculture International* 21, 927–937. <https://doi.org/10.1007/s10499-012-9561-1>.

- Funge-Smith, S.J., Briggs, M.R.P., 1998. Nutrient budgets in intensive shrimp ponds: implications for sustainability. *Aquaculture* 164, 117–133. [https://doi.org/10.1016/S0044-8486\(98\)00181-1](https://doi.org/10.1016/S0044-8486(98)00181-1).
- Gomes, L.C., Chagas, E.C., Martins Jr, H.M., Roubach, R., Ono, E.A., Lourenço, J.N.P., 2006. Cage culture of tambaqui (*Colossoma macropomum*) in Central Amazon floodplain lake. *Aquaculture* 253, 374–384. <https://doi.org/10.1016/j.aquaculture.2005.08.020>.
- Green, B.W., 1992. Water and chemical budgets for organically fertilized fish ponds in the dry tropics. Doctoral dissertation. Auburn University, Alabama, USA.
- Green, B.W., Boyd, C.E., 1995. Chemical budgets for organically fertilized fish ponds in the dry tropics. *Journal of the World Aquaculture Society* 26, 284–296. <https://doi.org/10.1111/j.1749-7345.1995.tb00257.x>.
- Gruca-Rokosz, R., Koszelnik, P., 2018. Production pathways for CH₄ and CO₂ in sediments of two freshwater ecosystems in south-eastern Poland. *PLoS one* 13, e0199755.
- Gruca-Rokosz, R., Tomaszek, J.A., Koszelnik, P., Czerwieńec, E., 2011. Methane and carbon dioxide fluxes at the sediment-water interface in reservoir. *Polish Journal of Environmental Studies* 20, 81–86.
- Hargreaves, J.A., 2006. Photosynthetic suspended-growth systems in aquaculture. *Aquaculture Engineering* 34, 344–363. <https://doi.org/10.1016/j.aquaeng.2005.08.009>.
- Hopkins, J.S., Sandifer, P.A., Browdy, C.L., 1994. Sludge management in intensive pond culture of shrimp—effect of management regime on water quality, sludge characteristics, nitrogen extinction, and shrimp production. *Aquaculture Engineering* 13, 11–30. [https://doi.org/10.1016/0144-8609\(94\)90022-1](https://doi.org/10.1016/0144-8609(94)90022-1).
- Jackson, C., Preston, N., Thompson, P.J., Burford, M., 2003. Nitrogen budget and effluent nitrogen components at an intensive shrimp farm. *Aquaculture* 218, 397–411. [https://doi.org/10.1016/S0044-8486\(03\)00014-0](https://doi.org/10.1016/S0044-8486(03)00014-0).
- Joynt, M.J., Kurup, B.M., Avnimelech, Y., 2011. Bioturbation as a possible means for increasing production and improving pond soil characteristics in shrimp-fish brackish water ponds. *Aquaculture* 318, 464–470. <https://doi.org/10.1016/j.aquaculture.2011.05.019>.
- Kimpara, J.M., Moraes-Valenti, P., Queiroz, J.F., New, M.B., 2013. Effects of intensification of the Amazon River prawn, *Macrobrachium amazonicum*, grow-out on effluent quality. *Journal of the World Aquaculture Society* 44, 210–219. <https://doi.org/10.1111/jwas.12021>.
- Kimpara, J.M., Tito Rosa, F.R., Preto, B.d.L., Valenti, W.C., 2011. Limnology of *Macrobrachium amazonicum* grow-out ponds subject to high inflow of nutrient-rich water and different stocking and harvest management. *Aquaculture Research* 42, 1289–1297. <https://doi.org/10.1111/j.1365-2109.2010.02717.x>.
- Koroleff, F., 1976. Determination of nutrients. In: Grasshoff, E., Kremling, E. (Eds.), *Methods of Seawater Analysis*. Verlag Chemie Weinheim, New York, NY, pp. 117–181.
- Lemonnier, H., Brizard, R., 1998. Effect of water exchange on pond ecosystem in shrimp semi-intensive rearing in New Caledonia during cold season. *Ifremer, Noumea, New Caledonia* 49.
- Levinski-Huf, F., Klein, V.A., 2018. Organic matter and physical properties of a Red Latosol under an integrated crop-livestock-forestry system. *Pesquisa Agropecuária Tropical* 48, 316–322. <https://doi.org/10.1590/1983-40632018v4852737>.
- Liu, L., Hu, Z., Dai, X., Avnimelech, Y., 2014. Effects of addition of maize starch on the yield, water quality and formation of bioflocs in an integrated shrimp culture system. *Aquaculture* 418, 79–86. <https://doi.org/10.1016/j.aquaculture.2013.10.005>.
- Lymer, D., Marttin, F., Marmulla, G., Bartley, D.M., et al., 2016. In: Taylor, W.W., Bartley, D.M., Goddard, C.I. (Eds.), *A global estimate of theoretical annual inland capture fisheries harvest*. Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 63–75.
- Mackereth, F.J.H., Heron, J., Talling, J.F., 1978. *Water Analysis: Some Revised Methods for Limnologists*, No. 36. Freshwater Biological Association. Titus Wilson and Son Ltd, Kendall.
- Mariscal-Lagarda, M.M., Páez-Osuna, F., 2014. Mass balances of nitrogen and phosphorus in an integrated culture of shrimp (*Litopenaeus vannamei*) and tomato (*Lycopersicon esculentum* Mill) with low salinity groundwater: A short communication. *Aquacultural Engineering* 58, 107–112. <https://doi.org/10.1016/j.aquaeng.2013.12.003>.
- Marker, A.F.H., Nusch, E.A., Rai, H., Riemann, B., 1978. The measurement of photosynthetic pigments in freshwaters and standardization of methods: Conclusions and recommendations. *Archives of Hydrobiologia* 14, 91–106.
- Marques, H.L.A., Moraes-Valenti, P.M.C., 2012. Current status and prospects of farming the giant river prawn *Macrobrachium rosenbergii* (De Man 1879) and the Amazon river prawn *Macrobrachium amazonicum* (Heller 1862) in Brazil. *Aquaculture Research* 43, 984–992. <https://doi.org/10.1111/j.1365-2109.2011.03032.x>.
- Marques, H.L.A., New, M.B., Boock, M.V., Barros, H.P., Mallasen, M., Valenti, W.C., 2016. Integrated Freshwater Prawn Farming: State-of-the-Art and Future Potential. *Reviews in Fisheries Science & Aquaculture* 24, 264–293. <https://doi.org/10.1080/23308249.2016.1169245>.
- Martin, J.L.M., Veran, Y., Guelorget, O., Pham, D., 1998. Shrimp rearing: stocking density, growth, impact on sediment, waste output and their relationships studied through the nitrogen budget in rearing ponds. *Aquaculture* 164, 135–149. [https://doi.org/10.1016/S0044-8486\(98\)00182-3](https://doi.org/10.1016/S0044-8486(98)00182-3).
- Matvienko, B., Sikar, E., Pinguelli Rosa, L., Dos Santos, M.A., De Filippo, R., Cimbliser, A.C.P., 2000. Gas release from a reservoir in the filling stage. *Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen* 27, 1415–1419. <https://doi.org/10.1080/03680770.1998.11901469>.
- Moraes-Valenti, P., Valenti, W.C., 2010. Culture of the Amazon river prawn *Macrobrachium amazonicum*. 485–501. In: New, M.B., Valenti, W.C., Tidwell, J.H., D'Abraham, L.R., Kutty, M.N. (Eds.), *Freshwater prawns: biology and farming*. Wiley-Blackwell, Oxford, England.
- Moraes-Valenti, P.M.C., Valenti, W.C., 2007. Effect of Intensification on Grow Out of the Amazon river prawn, *Macrobrachium amazonicum*. *Journal of the World Aquaculture Society* 38, 516–526. <https://doi.org/10.1111/j.1749-7345.2007.00125.x>.
- Moriarty, D.J., 1997. The role of microorganisms in aquaculture ponds. *Aquaculture* 151 (1–4), 333–349.
- Muthuwani, V., Kwei Lin, C., 1995. Water quality and nutrient budget in intensive shrimp culture ponds. *World Aquaculture*, vol. 96. World Aquaculture Society, Baton Rouge, LA, USA, pp. 270 Book of abstracts.
- Nhan, D.K., Verdegem, M.C., Milstein, A., Verreth, J.A., 2008. Water and nutrient budgets of ponds in integrated agriculture–aquaculture systems in the Mekong Delta. Vietnam. *Aquaculture Research* 39, 1216–1228. <https://doi.org/10.1111/j.1365-2109.2008.01986.x>.
- Páez-Osuna, F., Guerrero-Galván, S.R., Ruiz-Fernández, A.C., 1999. Discharge of nutrients from shrimp farming to coastal waters of the Gulf of California. *Marine Pollution Bulletin* 38, 585–592. [https://doi.org/10.1016/S0025-326X\(98\)00116-7](https://doi.org/10.1016/S0025-326X(98)00116-7).
- Páez-Osuna, F., Guerrero-Galván, S.R., Ruiz-Fernández, A.C., Espinoza-Ángulo, R., 1997. Fluxes and Mass Balances of Nutrients in a Semi-Intensive Shrimp Farm in North-Western Mexico. *Marine Pollution Bulletin* 34, 290–297. [https://doi.org/10.1016/S0025-326X\(96\)00133-6](https://doi.org/10.1016/S0025-326X(96)00133-6).
- Preto, B.L., Kimpara, J.M., Moraes-Valenti, P., Rosa, F.R.T., Valenti, W.C., 2011. Production strategies for short term grow-out of the Amazon River Prawn *Macrobrachium amazonicum* (Heller, 1862) in ponds. *Pan-American Journal of Aquatic Sciences* 6, 1–8.
- Ritvo, G., Dixon, J.B., Lawrence, A.L., Neill, W.H., Speed, M.F., 1998. Accumulation of chemical elements in Texas shrimp pond soils. *Journal of the World Aquaculture Society* 29, 422–431. <https://doi.org/10.1016/j.aquaculture.2004.09.013>.
- Sahu, B.C., Adhikari, S., Mahapatra, A.S., Dey, L., 2013. Carbon, nitrogen, and phosphorus budget in scampi (*Macrobrachium rosenbergii*) culture ponds. *Environmental Monitoring and Assessment* 185, 10157–10166. <https://doi.org/10.1007/s10661-013-3320-2>.
- Sahu, B.C., Adhikari, S., Mahapatra, A.S., Dey, L., 2015. Nitrogen, phosphorus, and carbon budgets in polyculture ponds of Indian major carps and giant freshwater prawn in Orissa State, India. *Journal of Applied Aquaculture* 27, 365–376. <https://doi.org/10.1080/10454438.2015.1115320>.
- Santos, M.J.M., Valenti, W.C., 2002. Production of Nile tilapia, *Oreochromis niloticus*, and freshwater prawn, *Macrobrachium rosenbergii*, stocked at different densities in polyculture systems in Brazil. *Journal of the World Aquaculture Society* 33, 369–376. <https://doi.org/10.1111/j.1749-7345.2002.tb00513.x>.
- Saraswathy, R., Muralidhar, M., Kailasam, M., Ravichandran, P., Gupta, B.P., Krishnani, K.K., Ponniah, A.G., Sundaray, J.K., Panigrahi, A., Nagavel, A., 2013. Effect of stocking density on soil, water quality and nitrogen budget in *Penaeus monodon* (Fabricius, 1798) culture under zero water exchange system. *Aquaculture Research* 44, 1578–1588. <https://doi.org/10.1111/j.1365-2109.2012.03165.x>.
- Sartory, D.P., Grobbelaar, J.U., 1984. Extraction of chlorophyll a from freshwater phytoplankton for spectrophotometric analysis. *Hydrobiologia* 114, 177–187. <https://doi.org/10.1007/BF00031869>.
- Silveira, G.A., Saran, L.M., Melo, W.J.D., Alves, L.M.C., 2016. Farming and soil urban occupation in the water quality of Jaboticabal and Cerradinho streams. *Ciência e Agrotecnologia* 40, 633–646. <https://doi.org/10.1590/1413-70542016406048415>.
- Sokal, R.R., Rohlf, F.J., 1995. *Biometry*, 3rd. Ed. Freeman, New York, pp. 887.
- Teichert-Coddington, D.R., Rouse, D.B., Potts, A., Boyd, C.E., 1999. Treatment of harvest discharge from intensive shrimp ponds by settling. *Aquacultural Engineering* 19, 147–161. [https://doi.org/10.1016/S0144-8609\(98\)00047-8](https://doi.org/10.1016/S0144-8609(98)00047-8).
- Thakur, D.P., Lin, C.K., 2003. Water quality and nutrient budget in closed shrimp (*Penaeus monodon*) culture systems. *Aquacultural Engineering* 27, 159–176. [https://doi.org/10.1016/S0144-8609\(02\)00055-9](https://doi.org/10.1016/S0144-8609(02)00055-9).
- Wahab, M.A., Bergheim, A., Braaten, B., 2003. Water quality and partial mass budget in extensive shrimp ponds in Bangladesh. *Aquaculture* 218, 413–423. [https://doi.org/10.1016/S0044-8486\(03\)00009-7](https://doi.org/10.1016/S0044-8486(03)00009-7).
- Woynárovich, A., van Anrooy, R., 2019. Field guide to the culture of tambaqui (*Colossoma macropomum*, Cuvier, 1816). *FAO Fisheries and Aquaculture Technical Paper* no. 624. Rome, Italy, 132pp.
- Wurts, W.A., Durborow, R.M., 1992. Interactions of pH, carbon dioxide, alkalinity and hardness in fish ponds. Southern Regional Aquaculture Center Publication v464, 4pp.