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Integrated multitrophic aquaculture in ponds using substrate for periphyton as natural source of food

Mariana Negri[1], Daiane Mompean Romera[2], Fabiana Garcia[3]*

[1]Universidade Estadual Paulista "Júlio de Mesquita Filho", Centro de Aquicultura – Jaboticabal (SP), Brazil.
[2]Instituto Agronômico de Campinas – Campinas (SP), Brazil.
[3]Instituto de Pesca – São Paulo (SP), Brazil.
*Corresponding author: fabiana.scaloppi@sp.gov.br

ABSTRACT

As a productive and sustainable alternative to fish farmers, the present study aimed to evaluate the use of substrate for periphyton growth in an integrated multitrophic aquaculture (IMTA) with complementary species. The studied species were: Nile tilapia (*Oreochromis niloticus*), grass carp (*Ctenopharyngodon idella*), and *curimbatá* (*Prochilodus lineatus*). The experiment had four treatments with three replicates each that evaluated the IMTAs: [T100] Cb:C – tilapia inside hapas fed on recommended feed, with grass carp and *curimbatás* outside the hapas making use of natural food; [T50] Cb:C – the same species distribution with tilapia fed 50% of the daily amount of commercial diet; and Cb:C 100 and Cb:C 50 – grass carp and *curimbatás* fed on recommended feed at two feeding rates (100 and 50%) with substrate for periphyton growth in the feeding restriction treatment. In phase II, tilapias were included in all the treatments as a complementary species. Growth performance of fish and physical-chemical parameters of water were evaluated. In the proposed models, the species were efficient in utilizing the feed/food and in nutrient cycling, achieving productivity of 7 t/ha in the system without water renewal. Inserting secondary and complementary species reduced the feed conversion ratio (FCR) to values of 0.95 in systems under feed restriction and 1.28 in the groups that received 100% of commercial feed. Considering the reduction of the FCR and the high productivity, farmers can diversify their products in the same area without increasing inputs.

Keywords: *Oreochromis niloticus*; *Ctenopharyngodon idella*; *Prochilodus lineatus*; Sustainability.

Aquicultura multitrófica integrada em viveiros escavados usando substrato para perifíton como fonte de alimento natural

RESUMO

Como uma alternativa produtiva e sustentável ao piscicultor, o presente trabalho teve como objetivo avaliar o uso de substrato para o crescimento do perifíton em um cultivo multitrófico (IMTA) com espécies complementares em viveiros escavados. As espécies estudadas foram: tilápia-do-nilo (*Oreochromis niloticus*), curimbatá (*Prochilodus lineatus*) e carpa-capim (*Ctenopharyngodon idella*). O experimento contou com quatro tratamentos e três repetições cada um, sendo testados os sistemas multitróficos integrados (IMTA): [T100] Cb:C – tilápias-do-nilo em hapas alimentadas com ração comercial, com curimbatás e carpas-capim soltas fora do hapa, aproveitando o alimento natural; [T50] Cb:C – mesma distribuição de espécies, sendo a tilápia alimentada com 50% da ração comercial; e Cb:C 100 e Cb:C 50 – curimbatás e carpas-capim soltas no viveiro, alimentados com duas taxas de arraçoamento (100 e 50% da ração), com a adição de substratos para o crescimento de perifíton no tratamento com a restrição alimentar. Na fase II, foram inseridas tilápias-do-nilo como espécie complementar em todos os tratamentos. O desempenho produtivo dos animais e os parâmetros físico-químicos da água foram avaliados. Nos modelos propostos, as espécies foram eficientes em aproveitar o alimento oferecido e na ciclagem de nutrientes, alcançando produtividade de 7 t/ha em sistema sem renovação de água. A inserção das espécies secundárias e complementares diminuiu a conversão alimentar para valores de até 0,95 em sistemas com restrição alimentar e 1,28 em cultivo com 100% da ração comercial. Com a redução da conversão alimentar e o aumento da produtividade, o piscicultor é capaz de diversificar seus produtos em uma mesma área sem o acréscimo de insumos.

Palavras-chave: *Oreochromis niloticus*; *Ctenopharyngodon idella*; *Prochilodus lineatus*; sustentabilidade.

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INTRODUCTION

In order to reach high production levels, the aquaculture industry invests in highly fed monocultures, primarily aiming at weight gain and productivity. In these systems, among the entire feed offered in the diet, only 20% is converted into biomass, leaving 80% of the material that is lost or incorporated into non-target biota (Valenti et al., 2011). Nonetheless, modern aquaculture faces the challenges of remaining, at the same time, efficient and highly productive, using the minimum of resources to produce as much food as possible, without causing waste (Edwards et al., 2016).

In this sense, periphyton-based aquaculture can solve this challenge. Periphyton is a natural food with high levels of protein, vitamins, and minerals. It is composed by a community attached to a substrate comprising algae, rotifers, cladocerans, protozoa filamentous bacteria, in addition to inorganic components (Moschini-Carlos, 1999; Wetzel, 1983). The periphytic community forms the basis for many food chains, serving as food for various aquatic organisms, including some that are commercially important, such as tilapia (*Oreochromis* sp.) (David et al., 2022a, 2022b; Garcia et al., 2016; Garcia et al., 2017; Huchette et al., 2000; Ibrahim et al., 2023; Moraes et al., 2020; Moschini-Carlos, 1999).

In some environments, periphyton is responsible for up to 90% of primary production (Wetzel, 1990). The periphyton has been used in aquaculture to complement the diet and reduce inputs without interfering with the performance of the species. In organic aquaculture, including substrates appears as an alternative, and the use of substrates occupying 30–40% of the pond area reduces 30–40% of the inputs spent on feeding at the density of 1.5 fish/m^2 (Milstein et al., 2005; Milstein et al., 2013). In Nile tilapia monoculture effluent, Nile tilapia can be produced without commercial feed inserting substrates for periphyton growth (David et al., 2022b).

In this context of ecological intensification, to maximize the use of nutrients, integrated multitrophic aquaculture (IMTA) is a promising alternative. The IMTA concept is extremely flexible, its cultivation is variable, and the IMTA can be applied to open water, continental waters, and variations of both (Barrington et al., 2009). However, it converges by using species from different trophic levels that occupy different niche spaces and complement each other in the ecosystem, to convert waste from the feed offered to the target species as fertilizer, energy, and food for the other species in the cultivation (Biswas et al., 2019; Chopin et al., 2012; Dantas et al., 2019; David et al., 2017; Flickinger et al., 2019; Flickinger et al., 2020a; Flickinger et al., 2020b; Franchini et al., 2020; Henry-Silva et al., 2023; Marques et al., 2021). The use of substrates in continental IMTA has not been studied extensively (Ibrahim et al., 2023). In a study evaluating the use of substrates in the culture of Malaysian shrimp (*Macrobrachium rosenbergii*) and Nile tilapia (*Oreochromis niloticus*), an improvement of 56% in the Nile tilapia yield was observed, along with 30% greater weight gain (Uddin et al., 2009).

Therefore, the present study aimed to test the zootechnical viability of using the natural food of an integrated multitrophic culture with species used by Brazilian fish farmers (grass carp, *Ctenopharyngodon idella*; *curimbatá*, *Prochilodus lineatus*; and Nile tilapia, *O. niloticus*).

MATERIAL AND METHODS

The present study was carried out at the Centro de Seringueira e Sistemas Agroflorestais of the Instituto Agronômico, in Votuporanga, SP, Brazil, in six ponds of 96-m^2 water surface and 1.3-m water depth divided longitudinally with a 2-m-high screen and 5-mm mesh, totaling 12 experimental units. For the beginning of the experiment, the ponds were fertilized with cattle manure, approximately 10 kg per experimental area, totaling 20 kg per pond.

The ponds were maintained without water renewal, only replacing evaporated/infiltrated water. This water supply came from a dam supplied by own spring, and, due to the low rainfall in 2020, from the fourth month of the experiment, water was pumped from dam to ponds. To prevent the entry of predators and/or fish larvae, a 300-µm mesh filter was installed on each water inlet of pond.

The experiment lasted the total of 222 days (March to November 2020) and was divided into two phases. Phase I lasted 160 days, and phase II, 62 days. The fingerlings and juveniles of fish were acquired from commercial fish farms, and the initial weight were: 45.5 g ± 2.7; *curimbatás* (*P. lineatus*), 2.7 g ± 0.4; and grass carp (*C. idella*), 23.3 g ± 2.7.

Experimental design

Phase I

The experiment was carried out in a completely randomized design with four treatments and three replications each. The treatments were two IMTA models and two feeding managements (50 and 100% feeding) (Fig. 1):

- IMTA [T100] Cb:C: Nile tilapia (main species) in hapas fed with 100% of the diet recommended by the fish feed industry. *Curimbatás* (secondary species) and grass carp (complementary species) stocked outside of the hapa, consumption the natural food (periphyton from the hapa screen, planktonic organisms from the water column and benthic organisms from bottom pond);
- IMTA [T50] Cb:C: Nile tilapia in hapa (main species) fed with 50% of the diet recommended by the fish feed industry.

Curimbatás (secondary species) and grass carp (complementary species) stocked outside of the hapa, consumption the natural food (periphyton from the hapa screen, planktonic organisms from the water column and benthic organisms from bottom pond);

- IMTA Cb:C-100 (without substrate): *curimbatás* (main species) and grass carp (secondary species) fed with 100% of the recommended feed;
- IMTA Cb:C-50 (with substrate): *curimbatás* (main species) and grass carp (secondary species) fed with 50% of the recommended feed and using substrates for periphyton growth.

Phase II

At the end of phase I, Nile tilapia was included as a complementary species in all treatments. The fish started the second phase with an average weight of 350.71 g ± 91.4 for Nile tilapia, 55.08 g ± 20.5 for *curimbatás* and 126.02 g ± 44.1 for grass carp. The treatments were: IMTA [T100] Cb:C:T and IMTA [T50] Cb:C:T with Nile tilapia fed inside hapa (main species), *curimbatás* (secondary species), and grass carp and Nile tilapia (complementary species) with no access to fish feed. The IMTA Cb:C:T-100 and IMTA Cb:C:T-50 comprised *curimbatás* (main species), and grass carp and Nile tilapia (secondary species) fed with commercial feed by the amount of each treatment (100 and 50%).

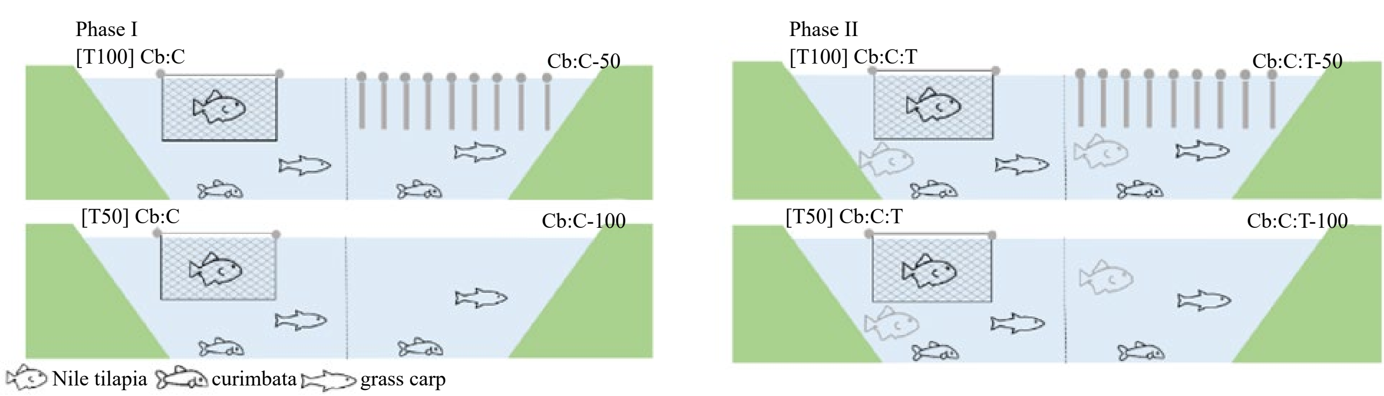
The treatments were distributed within the blocks (ponds divided in half, longitudinally). Thus, each pond contained a treatment with 100% of the feed and another with 50% of feed (Fig. 1).

The proportion between each species stocked followed the recommendations proposed by Casaca et al. (2005), which encourages the choice of a main species that enters the system in greater quantity (50 to 100%), followed by secondary species (20 to 30%) with the role of taking advantage of excess organic matter in the system and, finally, the complementary species (5 to 10%), in smaller quantities, that can take advantage of the natural food remaining from the primary and secondary species. Thus, the treatments, according to the proportions, are shown in Table 1.

In the Cb:C-50 and Cb:C:T-50 treatments, nine substrate panels (1×1.5 m) were placed for periphyton growth (Fig. 2). These were made of polyvinyl chloride (PVC) pipes on the upper and lower edges and a white shade screen with 5-mm^2 nylon mesh in the panel area. The upper pipe served as a float and the lower one as a counterweight, which was filled with crushed stone to keep the substrate submerged.

In treatments [T] Cb:C and [T] Cb:C:T, Nile tilapia were placed in 3 × 1-m hapas, made with PVC pipes, and 5-mm^2 nylon mesh on the upper and lower edges (Fig. 2). In all experimental units, the two sides of the screen (panels or hapas) sum, corresponded to 50% of the pond area.

Different commercial diets were used to feed the target species during the experiment. In phase I, for the *curimbatás* and grass carps (Cb:C-50 and Cb:C-100) an extruded diet with 40% of crude protein (CP) and 5% of lipid (2-mm pellets) was offered during the first 90 days of the experiment. Nile tilapia ([T50] Cb:C and [T100] Cb:C) was fed an extruded diet with 36% of CP and 6% of lipid (4-mm pellets) in the same period. After this period, which comprised phase II of the experiment and up to the end, extruded feed with 32% CP and 6% of lipid was used, initially with 4-mm pellets and, later, 6 mm for both of the main species.



IMTA: integrated multitrophic aquaculture.

Figure 1. Distribution scheme of the experimental design. In phase I, IMTA [T100] Cb:C treatment (treatment with Nile tilapia inside the hapa fed with 100% of the commercial feed with *curimbatás* and grass carp in the surroundings) and IMTA Cb:C-50 (treatment with *curimbatás* and grass carp fed 50% of the feed including nine substrate panels for periphyton growth), IMTA [T50] Cb:C (Nile tilapia inside the hapa fed 50% of the feed with *curimbatás* and carp grass in the surroundings) and IMTA Cb:C-100 (*curimbatás* and grass carp fed 100% of the feed). In phase II, Nile tilapia was included as a complementary species in all treatments.

Table 1. Proportion of distribution of each species, stocking density in the studied IMTAs, in phases I and II of the experiment.

	IMTA [T]Cb:C (%)	IMTA Cb:C (%)
Phase I		
Nile tilapia (hapa)[1]	88 (64)	-
Curimbatá[1]	33 (28)	66 (80)
Grass carp[1]	9 (8)	16 (20)
Stocking density[2]	2.24	1.2
Phase II		
Nile tilapia (hapa)[1]	30 (42)	-
Curimbatá[1]	20 (30)	40 (56)
Grass carp[1]	9 (14)	16 (22)
Nile tilapia (free in the pond) [1]	9 (14)	16 (22)
Stocking density[2]	1.13	1.2

IMTA: integrated multitrophic aquaculture; [1]number of fish (%); [2]fish/m^2.

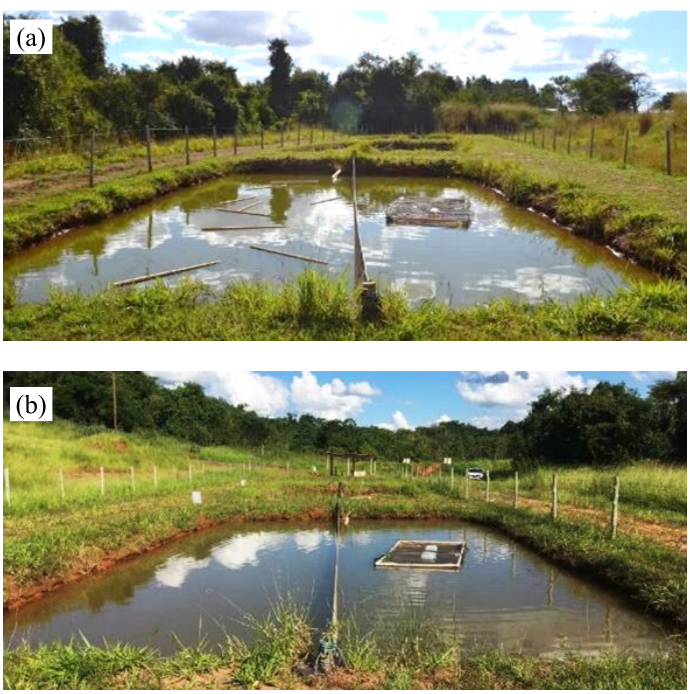


Figure 2. Arrangement of the hapas and substrate panels of the treatments during the experiment. (a) treatments [T50] Cb:C and Cb:C-100 treatments; (b) treatments [T100] Cb:C and Cb:C-50.

The daily portion was calculated through biweekly biometrics and the feed manufacturer indication. The amount of feed offered was divided into three daily portions in the first 45 days for treatments with 100% of the commercial feed and two daily portions for treatments with 50% of the feed. Then, after the first 45 days, the daily portions were reduced to two daily feeding for treatments with 100% feed and one feeding for treatments with 50% of the recommended daily feeding.

Evaluated parameters

Water quality

For characterization of the water ponds, these parameters were measured weekly: temperature (°C), dissolved oxygen (DO; mg/L), and electrical condutivity (µS/cm), using an Akso-AK88 multiparameter probe at two different times: 8 a.m. and 4 p.m. In treatments [T] Cb:C and [T] Cb:C:T, the probe was inserted into the hapas at a depth between 20 and 30 cm from the surface. In treatments Cb:C and Cb:C:T, the probe was inserted at the same depth in the pond area. The transparency was measured once a week using a Secchi disk.

Analyses of water nutrients (ammonia, nitrite, nitrate, and total phosphorus) were performed in each treatment, respecting the division of the ponds. Moreover, from each treatment, 500 mL of water, were collected at a depth between 20 and 30 cm and stored in plastic bottles, which were frozen for the analyses of water nutrients (ammonia, nitrite, nitrate, and total phosphorus. The collections were made at the beginning of the experiment, in the transition from phase I to phase II and at the end of the trial, on days 1, 155 and 222, respectively. At the end of the experiment, the samples were analyzed according to the methodology described by American Public Health Association (2005) (Table 2; Figs. 3 and 4).

Zootechnical performance

At the beginning and at the end of the experiment, individual biometrics of 5% of the fish in each experimental unit were performed. Biweekly, biometrics were performed of random samples with at least 20% of the total individuals of each treatment for feed adjustment. With these values, the weight gain (WG), produced biomass, and feed conversion ratio (FCR) were calculated, following Eqs. 1, 2 and 3:

$$\text{WG (g)} = \text{Average final weight} - \text{Average initial weight} \quad (1)$$

$$\text{Productivity (kg)} = (\text{Final biomass} - \text{Initial biomass})/\text{Pond surface area} \quad (2)$$

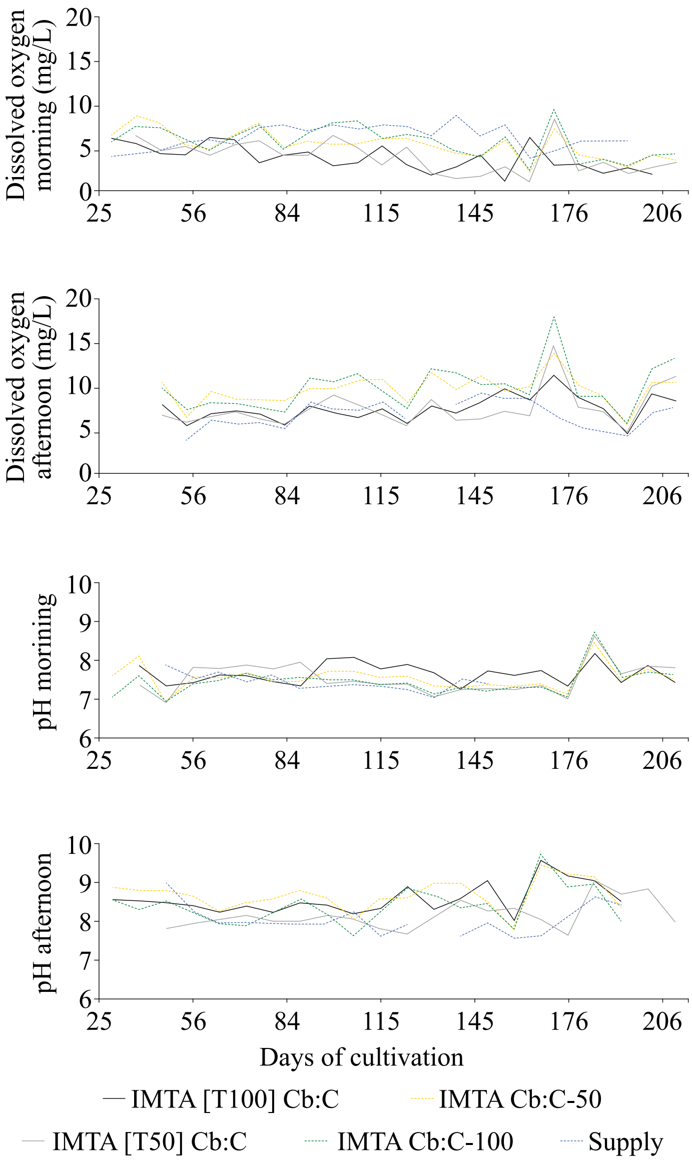
$$\text{FCR} = \text{GP} / \text{Feed consumption} \quad (3)$$

Every morning, we verified dead and/or dying fish to calculate the mortality during the experiment.

Table 2. Water quality variables during the two phases of the experiment*.

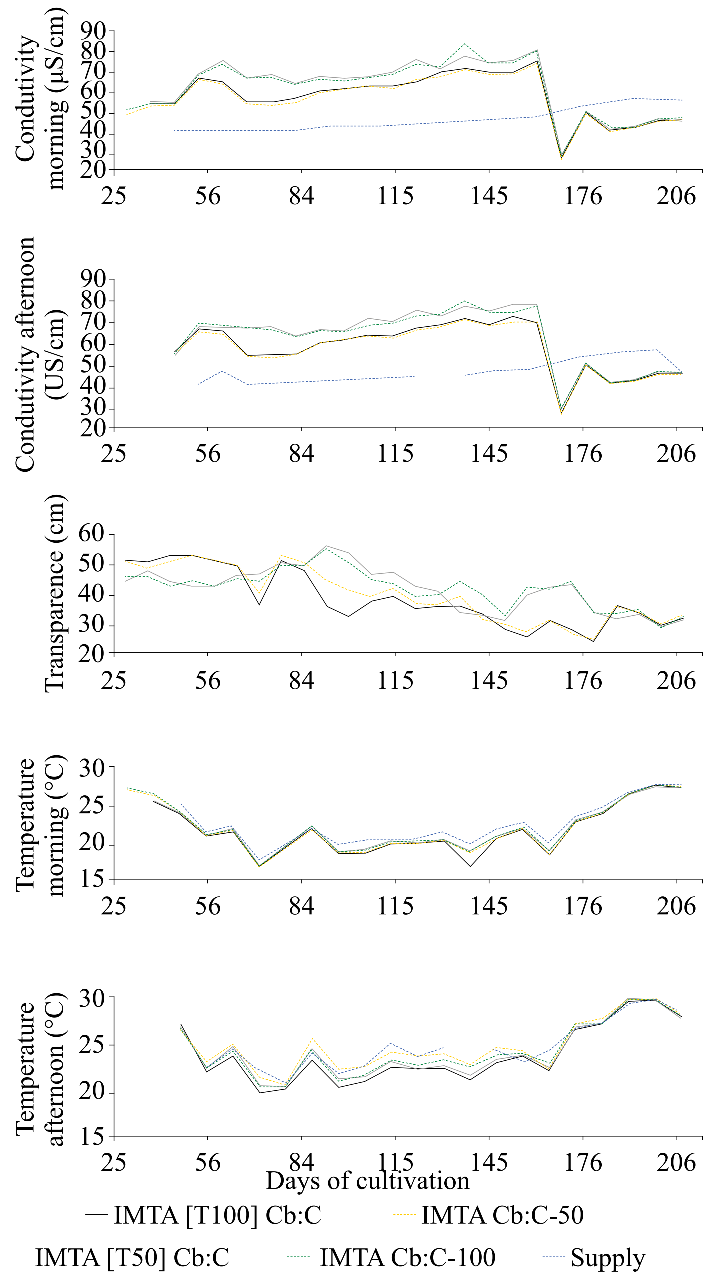
Phase I	**[T50]Cb:C**	**Cb:C-100**	**[T100]Cb:C**	**Cb:C-50**	**Water Supply**
Phase II	**[T50]Cb:C:T**	**Cb:C:T-100**	**[T100]Cb:C:T**	**Cb:C:T-50**	
Temperature morning (°C)					
Phase I	21.2 ± 2.0	21.5 ± 2.5	20.8 ± 2.2	21.4 ± 2.5	21.5 ± 1.7
Phase II	25.7 ± 1.7	25.7 ± 1.7	25.7 ± 1.9	25.7 ± 1.8	26.1 ± 1.7
Temperature afternoon (°C)					
Phase I	23.8 ± 2.7	24.4 ± 2.6	23.3 ± 2.9	24.5 ± 2.5	23.8 ± 1.5
Phase II	28.5 ± 1.2	28.6 ± 1.2	28.3 ± 1.3	28.6 ± 1.1	28.3 ± 1.3
DO morning (mg/L)					
Phase I	6.4 ± 1.6b	6.4 ± 1.5a	4.5 ± 1.4b	6.1 ± 1.5a	6.8 ± 1.3
Phase II	4 ± 2.5	4.7 ± 2.4	3.4 ± 1.7	4.4 ± 1.7	6.0 ± 1.1
DO afternoon (mg/L)					
Phase I	7.0 ± 1.0b	9.4 ± 1.6a	7.4 ± 1.2b	9.6 ± 1.3a	7.1 ± 1.6
Phase II	9.5 ± 4.1	10.2 ± 3.9	8.3 ± 2.3	9.9 ± 2.7	6.4 ± 1.6
pH morning					
Phase I	7.4 ± 0.3	7.3 ± 0.3	7.6 ± 0.6	7.5 ± 0.4	7.4 ± 0.2
Phase II	7.7 ± 0.7	7.6 ± 0.5	7.6 ± 0.4	7.7 ± 0.7	7.6 ± 0.4
pH afternoon					
Phase I	8.1 ± 0.2b	8.3 ± 0.3ab	8.4 ± 0.3a	8.6 ± 0.3a	8.0 ± 0.4
Phase II	8.4 ± 0.8	8.6 ± 0.7	8.9 ± 0.7	8.8 ± 0.7	8.2 ± 0.4
Condutivity (µS/cm)					
Phase I	69.3 ± 7.6	68.6 ± 7.3	64.4 ± 6.6	62.7 ± 6.4	44.7 ± 2.5
Phase II	49.0 ± 16.3	49.2 ± 16.2	47.7 ± 13.9	47.4 ± 13.6	54.6 ± 3.3
Transparency (cm)					
Phase I	43.7 ± 6.7	43.9 ± 5.3	39.7 ± 9.3	41.4 ± 9.0	> 50.0
Phase II	32.4 ± 5.2	31.6 ± 5.1	31.6 ± 6.8	31.9 ± 6.8	30.6 ± 5.6
Ammonia (mg/L)					
Phase I	0.066 ± 0.03		0.076 ± 0.04		0.060 ± 0.02
Phase II	0.075 ± 0.03		0.079 ± 0.05		0.046 ± 0.02
Nitrite (mg/L)					
Phase I	0.011 ± 0.04		0.004 ± 0.02		0.003 ± 0.04
Phase II	0.015 ± 0.02		0.004 ± 0.01		0.003 ± 0.00
Nitrate (mg/L)					
Phase I	0.003 ± 0.01		0.001 ± 0.00		0.001 ± 0.00
Phase II	0.004 ± 0.01		0.001 ± 0.00		0.001 ± 0.00
Total phosphorus (mg/L)					
Phase I	0.105 ± 0.09		0.122 ± 0.13		0.018 ± 0.01
Phase II	0.174 ± 0.02		0.192 ± 0.07		0.046 ± 0.03

DO: Dissolved oxygen; *means followed by different letters on the line differ from each other (Tukey's test; $p < 0.05$).



IMTA: integrated multitrophic aquaculture; DO: dissolved oxygen in the morning and in the afternoon.

Figure 3. Water quality variables over the experimental period. Dashed line delimits the experiment phases. After 160 days, Nile tilapia were inserted into IMTAs and phase II began, changing the nomenclature of treatments. IMTA [T] Cb:C became IMTA [T] Cb:C:T and IMTA Cb:C became IMTA Cb:C:T, keeping the same feeding rates.



IMTA: integrated multitrophic aquaculture.

Figure 4. Water quality variables over the experimental period. Dashed line delimits the experiment phases. After 160 days, Nile tilapia were inserted into IMTAs and phase II began, changing the nomenclature of treatments. IMTA [T] Cb:C became IMTA [T] Cb:C:T and IMTA Cb: C became IMTA Cb:C:T, keeping the same feeding rates.

Periphyton

During the experiment, an area of periphyton (532 cm^2) on screen was collected to evaluate the dry matter weight. Samples were collected bimonthly at a depth between 40 and 50 cm from the hapas and substrate panels, stored in 200-mL plastic pots, and dried with forced air circulation at 60°C, during 24 to 48 hours, until constant weight. After drying, the material was weighed on a Denver APX -200 analytical balance to determine the dry matter.

Statistical analysis

Statistical analysis was performed in StatSoft Statistica 7.0 and submitted to Shapiro–Wilk test on residuals test for normality, Levene's test for homogeneity of variance and analysis of variance (ANOVA). Initially, the similarity of the experimental conditions of water quality between treatments grouped in a single pond was evaluated (Group 1: [T100] Cb:C and Cb:C-50; and Group 2: [T50] Cb:C and Cb: C-100). The analysis of variance confirmed the similarity of the two groups with no significant difference ($p > 0.05$) for all water quality parameters evaluated in three periods: beginning, end and average of the whole period.

Based on this finding, the ANOVA was applied comparing the four treatments (two IMTAs and two feeding managements) for all performance and water quality parameters, and, when a significant difference was observed ($p < 0.05$), the means were compared by Tukey's test ($p < 0.05$). Each fish species was independently compared between treatments. There was no comparison of species within each treatment.

For the dry matter of periphyton accumulated in the substrates, ANOVA was applied, followed by the Tukey's test (5%), to compare the averages of the treatments ([T50] Cb:C, [T100] Cb:C, Cb:C-50 and Cb:C-100) within each assessment time (start of phase I, transition phase I to phase II, end of phase II).

RESULTS

Productive performance

Phase I

Nile tilapia from treatment [T100] Cb:C received twice as much feed as Nile tilapia from treatment [T50] Cb:C and had a weight gain 48% higher than [T50] Cb:C. As a consequence, the feed conversion of the feed restriction treatment was 36% lower than the [T100] Cb:C. In this IMTA model with Nile tilapia in hapas ([T100] Cb:C and [T50] Cb:C), the weight gain of unfed species was higher than IMTAs without the presence of Nile tilapia (Cb:C-50 and Cb:C-100), in which *curimbatás* and grass carp received commercial feed. For grass carp, the greatest weight gain was observed in [T50] Cb:C, which presented weight gain 2.4 times greater than Cb:C-50, 45% higher than [T100] Cb:C and 23% greater than Cb:C-100 (Fig. 5).

Feed conversion rate was higher in the Cb:C IMTA when compared to the treatments employing tilapia in hapas. In the [T]Cb:C IMTAs, the presence of *curimbatás* and grass carp increased the productivity of these IMTAs and reduced the FCR by 17% in the treatment [T50] Cb:C and 30% in the [T100] Cb:C compared to the FCR of Nile tilapia (Table 3).

Phase II

The best zootechnical performance of *curimbatás* and grass carps with Nile tilapias in phase I led us to include Nile tilapias in phase II as a complementary species in all the IMTAs studied.

Fish gained similar biomass in both phases, although phase I lasted 2.6 times longer than phase II. The weight gain of Nile tilapia fed on [T100] Cb:C:T was 37% higher than [T50] Cb:C:T that received half the recommended feed. As a consequence, the feed conversion of the feed restriction treatment was 17% lower compared to [T100] Cb:C:T (Figs. 3 and 4 and Table 2). In the IMTAs [T] Cb:C:T, the unfed Nile tilapia showed the same weight gain as those fed with 50% of the hapa diet (Fig. 5).

In the Cb:C:T-100 treatment, the Nile tilapia gained 415 g in 62 days, and, in the Cb:C:T-50 treatment, the weight gain was of 315 g similar to the Nile tilapia fed in the hapas with 100% of the recommended feed ([T100] Cb:C:T) (Figs. 3 and 4). The presence of Nile tilapia in all IMTAs resulted in a similar weight gain of *curimbatás* in all treatments, with a statistical difference between the Cb:C:T-100, with the highest value, and the Cb:C:T-50, presenting the lowest weight gain (Fig. 5).

The IMTAs studied showed a final productivity of up to 7 t/ha without water renewal (Table 2). The FCR was drastically reduced to values up to 0.95 in treatments with 50% of the feed restriction and 1.28 in the treatments fed with 100% of the recommended feed (Table 3).

Periphyton

The highest apparent periphyton colonization was observed in IMTAs [T]Cb:C, in which this natural food was fixed on the hapa walls (Fig. 6), with higher values than the Cb:C-50 treatment during the transition from phase I to phase II up to the end of the experiment.

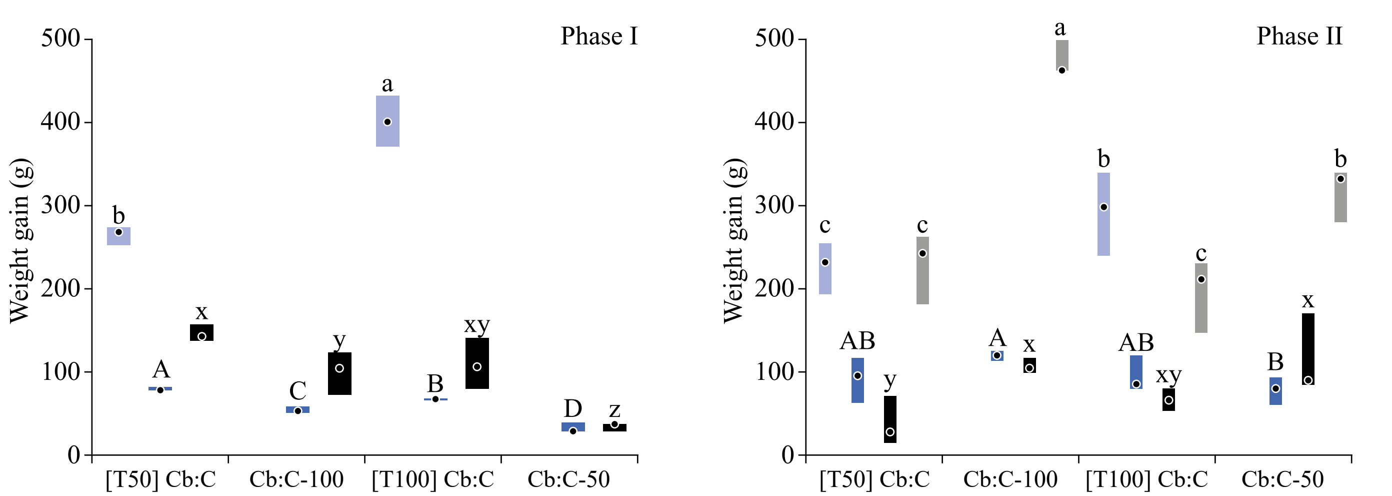
DISCUSSION

There were reduced mortality during the experiment and no difference between treatments. Much of the first phase of cultivation was carried out during the autumn and winter seasons, with temperatures below the appropriate temperature, mainly for Nile tilapia (Cudmore and Mandrak, 2004; Moura et al., 2007; Sverlij et al., 1993). For this reason, the increase in temperature in the second phase contributed to a better

Table 3. Indicators of zootechnical performance of treatments during the two phases of the experiment*.

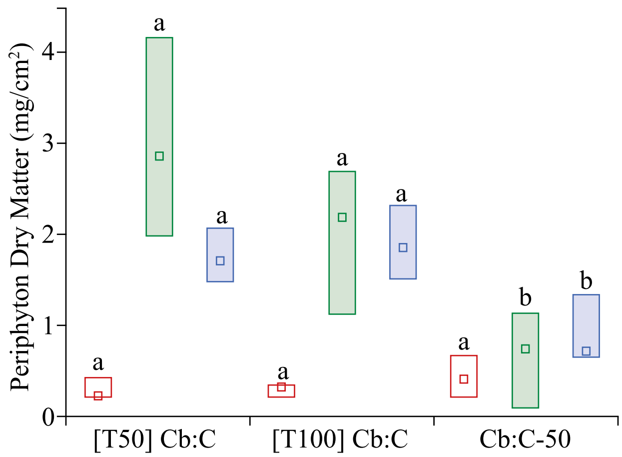
	Phase I			
	[T50] Cb:C	**Cb:C-100**	**[T100] Cb:C**	**Cb:C-50**
Productivity (kg/m^2)	0.71 ± 0.02^{a}	0.11 ± 0.01^{b}	0.71 ± 0.03^{a}	0.06 ± 0.01^{c}
Stocking density (fish/m^2)	2.9	1.6	2.3	1.6
Survival (%)	97.4 ± 0.02	100	98.5 ± 0.05	100
FCR of all species	0.94 ± 0.03^{a}	1.7 ± 0.4^{b}	1.15 ± 0.05^{a}	1.61 ± 0.05^{b}
FCR of Nile tilapia (hapa)	1.1 ± 0.02^{a}	-	1.5 ± 0.1^{b}	-
	Phase II			
	[T50] Cb:C:T	**Cb:C:T-100**	**[T100] Cb:C:T**	**Cb:C:T-50**
Productivity (kg/m^2)	0.44 ± 0.01^{b}	0.57 ± 0.01^{a}	0.43 ± 0.01^{b}	0.34 ± 0.01^{c}
Stocking density (fish/m^2)	1.3 ± 0.01	1.5	1.3 ± 0.01	1.5
Survival (%)	95.7 ± 7.1	100	94.5 ± 2.06	100
FCR of all species	0.94 ± 0.06^{a}	1.29 ± 0.06^{b}	1.28 ± 1.14^{b}	0.96 ± 0.06^{a}
FCR of Nile tilapia (hapa)	1.57 ± 0.22^{a}	-	1.84 ± 0.27^{b}	-

*Data are presented as mean ± standard deviation. Different letters show significant differences between treatments. Phase I: integrated multitrophic aquaculture (IMTA) [T] Cb:C with Nile tilapia in hapas and *curimbatás* and grass carp without food in the surroundings ([T100] Cb:C = 100% of the feed and [T50] Cb:C = 50% of the feed) and IMTA Cb:C with *curimbatás* and grass carps with commercial feeding (Cb:C-100 = 100% of the feed and Cb:C-50 = 50% of the feed) and the insertion of substrates in the treatment with food restriction. Phase II: insertion of Nile tilapia as a complementary species in IMTAs; FCR: feed conversion ratio.



■ Nile tilapia (hapa); ■ *curimbatá*; ■ grass carp; ■ Nile tilapia; • circles inside the rectangles are medians, and the rectangles are quartiles that show the dispersion (variability) of each species fish weight; phase I composition: IMTA [T] Cb:C with Nile tilapia in hapas and *curimbatás* and grass carp without feeding to the surroundings with two feed rates ([T100] Cb:C = 100% of the feed and [T50] Cb:C = 50% of the feed) and IMTA Cb:C with *curimbatás* and grass carps fed with two feeding rates (Cb:C-100 = 100% of the feed and Cb:C-50 = 50% of the feed) and the insertion of substrates in the treatment with food restriction; phase II composition: IMTA [T] Cb:C:T with Nile tilapia in hapas and *curimbatás*, grass carp and Nile tilapia without feeding to the surroundings with two feeding rates ([T100] Cb:C:T = 100% of the feed and [T50] Cb:C:T = 50% of the feed) and IMTA Cb:C:T with *curimbatás*, grass carp and Nile tilapia fed with two feed rates (Cb: C:T-100 = 100% of the feed and Cb:C:T-50 = 50% of the feed) and the insertion of substrates in the treatment with food restriction; comparison of means performed independently for each species. Means followed by different letters on the line differ from each other (Tukey's test; $p < 0.05$). Letters a, b, c and d compare Nile tilapia; letters A, B, C and D compare *curimbatás*, and letters x and y compare grass carp.

Figure 5. Weight gain obtained in the proposed integrated multitrophic aquaculture models.



Initial phase I; transition from phase I to phase II; phase II; *circles inside the rectangles are medians and the rectangles are quartiles, and both show the dispersion (variability) of each species fish weight. Different letters represent the statistical difference ($p < 0.05$) between treatments within each evaluation time (different colors).

Figure 6. Periphyton dry matter (mg/cm^2) during the experiment. Treatments [T50] Cb:C and [T100] Cb:C refer to the hapas colonization and Cb:C-50 to the panel colonization*.

development of the studied species, since the biomass gain was similar in both phases, although phase I lasted 2.6 times longer than phase II.

In the first phase, the *curimbatás* showed low growth in the Cb:C treatments, in which commercial feed was offered. In this aquaculture system, the species presented higher feed conversion values, indicating the low capacity to take advantage of the feed. In a study with different percentages of crude protein (CP) in commercial diets for juvenile of *curimbatá* (*Prochilodus scrofa*), diets with 44% of CP offered better conditions for the development of the species (Bernardes and Públio, 2012). Moreover, in this study, at 150 days, the *curimbatás* average weight was 43.49 g, below that achieved of fish from treatments [T] Cb:C (*curimbatás* without food) 127,6 and 147,6 g and Cb:C-100 137,1 g. Therefore, these results indicate that *curimbatá* should be used as a secondary or complementary species in an IMTA instead of the main species.

In the Cb:C-50 treatment, *curimbatás* did not have a good productive performance compared to the other treatments, probably because the substrates of this treatment showed low colonization since the beginning of the experiment (Fig. 6). In a natural environment, free-living *curimbatás* vary their diet according to the watershed where they are found. Among the main items consumed are periphyton, phytoplankton, sediment and soil microbial biomass, and algae (Benedito et al., 2018). Due to this ability to take advantage of natural food, *curimbatá* has been studied in polyculture systems or IMTAs. The use of this iliophagous species was evaluated in an integrated culture with *tambaqui* (*Colossoma macropomum*) and Amazonian shrimp (*Macrobrachium amazonicum*) to increase the total yield and improve the use of nutrients from the offered diet. Including *curimbatá* did not affect the development of *tambaqui* (Franchini et al., 2020). This shows the possibility of integrated cultivation with *curimbatá* without harming and improving the nutrient availability for other complementary species.

When Nile tilapia was stocked in Cb:C treatments pond as a complementary species in phase II, it assumed the role of main species in the IMTA, benefiting from the feed and not competing with the grass carp and *curimbatá* for natural food available. In this culture, Nile tilapia did the best weight gain compared to IMTAs [T] Cb:C:T, reared in hapas. In both phases of the experiment, *curimbatá* and grass carp performed better when reared with Nile tilapia and without fed. However, after stoking Nile tilapia outside the hapas at [T] Cb:C:T treatments, there was competition for natural food between grass carp and Nile tilapia. In this situation, only natural food was not enough for grass carp growth, resulting in low productive performance on second phase, when compared with Cb:C:T treatments. These results indicate that grass carp can be produced with Nile tilapia, if the last one is raised in hapa/cage/net tanks, without contact of each other to, and avoid direct competition for space and food.

It is likely in IMTAs fish composition studied the grass carp's feeding habits did not favor it. Although it can feed on a wide variety of species such as Cladocera, Copepoda, small invertebrates, algae, nymphs, etc., grass carp prefers macrophytes or terrestrial plants if they are available (Chilton and Muoneke, 1992; Edwards, 1973). Thus, our hypothesis was that Nile tilapia had an advantage over the grass carp for periphyton competition.

Based on the results of the present study and on studies that analyzed supplementation of the grass carp diet with different protein sources and silages (Camargo et al., 2006; Costa et al., 2008; Nascimento et al., 2018; Sponchiado et al., 2018), we recommend including grass forage in IMTAs with grass carp. Feed supplementation with tifton grass silage for grass carp fed with extruded feed (30% CP) improved weight gain (23.1 g in 45 days) and apparent feed conversion (1.45) of this species (Nascimento et al., 2018). However, this management should be recommended with caution, because, when fed exclusively with forage, grass carp presented problems on nutrition and high mortality (Camargo et al., 2006; Satiro et al., 2021).

In the treatments with restriction feeding ([T50] Cb:C, [T50] Cb:C:T and Cb:C:T-50), it was able to supplement its diet with natural food, that can improve the feed conversion ratio. Nile tilapia is a true omnivore, as it has an efficient digestive system able of digesting small benthic animals, algae, and also detritus (Beveridge and Baird, 2000). The tilapia prefers periphytic algae over phytoplankton (Dempster et al., 1995).

CONCLUSION

Nile tilapia played an important role in providing nutrients and, consequently, in the production of natural food for the *curimbatás*. In the proposed IMTA systems, the secondary and complementary species (grass carp, *curimbatá* and Nile tilapia) proved to be efficient in taking advantage of natural food available in the environment through the diet of the main species (Nile tilapia in the hapa). Under these conditions, the IMTAs showed reduction in feed conversion with values of 0.95 in treatments with feed restriction and 1.28 in treatments with 100% of commercial feed.

The use of available natural food through the diet of the main species in the proposed system allowed productivity up to 7.1 t/ha without water renewal. The IMTAs studied proved to be advantageous for fish farmers, who can achieve good productivity and diversify their aquaculture species in the same area without increasing inputs.

CONFLICT OF INTERESTS

Nothing to declare.

ETHICAL APPROVAL

Approved by the Animal Experimentation Ethics Committee of the Instituto de Pesca (CEEAIP), on June 29, 2022.

DATA AVAILABILITY STATEMENT

All dataset were generated/analysed in the current study.

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AUTHORS' CONTRIBUTIONS

Conceptualization: Romera DM, Garcia F; **Data curation:** Negri M, Romera DM; **Formal Analysis:** Negri M; **Validation:** Romera DM, Garcia F; **Software:** Garcia F; **Supervision:** Garcia F; **Project administration:** Romera DM; **Funding acquisition:** Garcia F; **Writing – original draft:** **Writing – review & edition:** Romera DM, Garcia F; **Final approval:** Garcia F.

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