UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CENTRO DE AQUICULTURA DA UNESP

APLICAÇÃO DE INDICADORES E ÍNDICES PARA AVALIAR A SUSTENTABILIDADE AMBIENTAL EM UM SISTEMA DE AQUICULTURA INTEGRADO E MULTITRÓFICO, COM DIFERENTES SUBSTRATOS

Danilo Cintra Proença

Jaboticabal, São Paulo
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Orientador: Wagner Cotroni Valenti

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

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SUMÁRIO

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III. INTRODUÇÃO GERAL

Devido ao crescimento acelerado da população humana, a preocupação em suprir a demanda por alimentos aumentou. A aquicultura é uma atividade importante para contribuir com o fornecimento de alimentos em escala mundial (FAO, 2012). Porém, quando gerenciada de forma inadequada, gera impactos ambientais negativos que põem em dúvida sua sustentabilidade (Clay, 1997; Naylor et al., 1998, 2000). Os principais são: poluição de corpos d'água pelos efluentes produzidos, ineficiência do uso de recursos naturais, excessivo uso do solo e de água, escape de espécies exóticas introduzidas na aquicultura, entre outros (Boyd et al., 2003).

Na tentativa de direcionar a aquicultura para o crescimento sustentável, algumas estratégias estão sendo adotadas. Entre elas, o policultivo é um sistema em que duas ou mais espécies são cultivadas, em uma mesma instalação, e que vem ganhando destaque devido às vantagens em relação ao monocultivo (Uddin et al., 2006; New e Zimmermann, 2010). Geralmente, usam-se espécies com diferentes nichos ecológicos, permitindo melhor uso do espaço no sistema e o aproveitamento do alimento natural presente nos viveiros. Nesse caso, é chamado cultivo multiespacial e multitrófico (MMAS). O MMAS ainda pode ser melhorado com a introdução de substratos no sistema. Estes possibilitam a colonização de perifíton em sua superfície, disponibilizando maior quantidade de alimento natural aos animais. Além disto, promove uma ciclagem de nutrientes no sistema, tornando-o mais eficiente no aproveitamento de resíduos.

Teoricamente, os MMAS são mais adequados. No entanto, é necessário avaliar se os benefícios dessas estratégias realmente tornam os cultivos mais sustentáveis. Para isso, podemos utilizar uma ferramenta bastante usada para monitorar e avaliar os impactos ambientais que é o conjunto de indicadores (Warhurst, 2002). Eles fornecem valores numéricos que refletem cada parte do sistema, além de permitir uma comparação direta (Valenti et al., 2011). Assim, este trabalho utilizou esta ferramenta para gerar informações sobre a sustentabilidade ambiental dos sistemas MMAS de tilápia-do-nilo e camarão-da-amazônia, com adição de dois tipos de substrato: bambu e manta geotêxtil em viveiros experimentais. Essas informações podem contribuir para compreender impactos positivos e negativos dos sistemas e ajudar no desenvolvimento sustentável da aquicultura. Os resultados podem ajudar a entender a sustentabilidade de sistemas de policultivo e contribuir para o aprimoramento do uso de indicadores de sustentabilidade na aquicultura.

Essa Dissertação foi redigida sob a forma de artigo científico, que é apresentado a seguir. As citações e referências foram formatadas de acordo com as normas do periódico Ecological Indicators, ao qual pretendemos submetê-lo após a defesa.

IV. RESUMO

A aquicultura é a atividade de produção de alimentos que mais cresce no mundo e tem sido criticada por seus impactos ambientais negativos. O caminho em direção da sustentabilidade é incerto e precisa ser avaliado. Foi utilizado um conjunto de indicadores para avaliar a sustentabilidade em um sistema de aquicultura integrado e multitrófico, usando espécie bentônica e nectônica combinadas com dois tipos de substratos. Um estudo de 5 meses foi realizado utilizando três diferentes tratamentos em um delineamento inteiramente casualizado: controle, sem substrato (WS); substrato manto geotêxtil (GS), e substratos de bambu (BS). Densidade de estocagem foi de 22 camarões m^{-2} e 1,1 tilápias m^{-2} para os tratamentos. As tilápias foram alimentadas com ração, aproximadamente 5-1% de sua biomassa, de acordo com a fase de desenvolvimento. A sustentabilidade ambiental foi medida com base na utilização de recursos naturais, a eficiência na utilização de recursos, a poluição liberada para o meio ambiente e o risco da espécie cultivada. Um índice de sustentabilidade ambiental foi criado a fim de avaliar qual o tratamento era mais sustentável. Os resultados mostraram que a GS melhorou a utilização dos recursos e BS a melhor eficiência da utilização dos recursos, além de melhorar a qualidade do sedimento. Apesar das vantagens da utilização de substrato, WS foi considerado o sistema mais sustentável. O uso de substratos não aumentou a sustentabilidade, devido principalmente a questão de emissão de gases.

Palavras-chave: sustentabilidade, indicadores, policultivo, substrato, meio ambiente, camarão, tilápia.

V. ABSTRACT

Aquaculture is the food production activity that grows faster than any other in the world and has been criticized for its environmental negative impacts. The path toward sustainability requirements is uncertain and needs to be evaluated. We used a set of indicators to assess the sustainability of integrated aquaculture multispatial and multitrophic systems, using a benthonic and a nektonic omnivorous species combined with two types of substrates. A 5-month study was conducted using three different treatments in a completely randomized design: control, without substrate (WS); geotextile mantle substrate (GS); and bamboo substrate (BS). Stocking density was 22 prawns m^{-2} and 1.1 tilapias m^{-2} for all treatments. Tilapia was fed about 5-1% of their biomass, according to the development phase. Environmental sustainability was measured based on the use of natural resources, efficiency on the use of resources, pollution released to environment and risk of cultivated specie. An environmental sustainability index was created in order to evaluate which treatment was more sustainable. Results have shown that GS improved the use of resources and BS had the best efficiency of the use of resources and enhanced the sediment quality. Despite of the advantages of using substrate, WS was considered the most sustainable system. The use of substrates did not increase the sustainability due mainly to gas emission issue.

Keywords: sustainability, indicators, polyculture, substrate, environment, prawn, tilapia.

VI. ARTIGO

APPLICATION OF INDICES AND INDICATORS TO ASSESS ENVIRONMENTAL SUSTAINABILITY IN AN INTEGRATED AQUACULTURE MULTITROPHYC SYSTEM WITH DIFFERENT SUBSTRATES

ABSTRACT

Aquaculture is the food production activity that grows faster than any other in the world and has been criticized for its environmental negative impacts. The path toward sustainability requirements is uncertain and needs to be evaluated. We used a set of indicators to assess the sustainability of integrated aquaculture multispatial and multitrophic systems, using a benthonic and a nektonic omnivorous species combined with two types of substrates. A 5-month study was conducted using three different treatments in a completely randomized design: control, without substrate (WS); geotextile mantle substrate (GS); and bamboo substrate (BS). Stocking density was 22 prawns m^{-2} and 1.1 tilapias m^{-2} for all treatments. Tilapia was fed about 5-1% of their biomass, according to the development phase. Environmental sustainability was measured based on the use of natural resources, efficiency on the use of resources, pollution released to environment and risk of cultivated specie. An environmental sustainability index was created in order to evaluate which treatment was more sustainable. Results have shown that GS improved the use of resources and BS had the best efficiency of the use of resources and enhanced the sediment quality. Despite of the advantages of using substrate, WS was considered the most sustainable system. The use of substrates did not increase the sustainability due mainly to gas emission issue.

Keywords: sustainability, indicators, substrate, environment, polyculture, prawn, tilapia.

1. Introduction

Indicators have been widely used for monitoring and assessing several environmental impacts (Warhurst, 2002). They are simple measures most often quantitative that represent a state of economic, social and/or environmental development in a defined region (Ness et al., 2007). Moreover, indicators simplify, quantify, analyze and communicate the complex and complicated information by conceptualizing phenomena and assessing the trends and identifying the hotspots (Warhurst, 2002). Understanding these trends allows making short-term projections and relevant decisions for the future (Ness et al., 2007). Indicators provide values that can be combined in an index, allowing a simple and understandable interpretation of the complex meaning of sustainability and the tracking of long-term sustainability trends from a retrospective point of view (Ness et al., 2007; Valenti, 2008). The indicators are an important way to evaluate production systems or treatments in experiments (Valenti et al., 2011). Through this tool, we can compare today's aquaculture systems with others previously used, and make projections for the future. It may help to introduce new practices to modify the current weaknesses (problems) and improve the sustainability of the systems.

Aquaculture is an important activity to meet the growing population demand for food. This sector shows the fastest growth in the world, increasing ~8.8% per year and currently means 47% of the total seafood supply (FAO, 2012). This activity has grown significantly enough to cause negative impacts, and so it is usually criticized for environmentalists because of natural resources waste and potential environmental and social negative impacts (Clay, 1997; Naylor et al., 1998, 2000; Kutty, 2005). There are several concerns regarding aquaculture: water pollution from pond effluents, inefficient utilization of natural resources, excessive use of ground water and others freshwater supplies, escape of exotic species introduced for aquaculture and others (Boyd et al., 2003). In general, aquaculture is the rearing of one or more species in a complex ecosystem, which involves at least four compartments: water, sediment, atmosphere and the organisms (biodiversity). They interact and affect each other and are parts of the production. The efforts to achieve a more sustainable aquaculture must consider these compartments as a whole.

Polyculture is the farming of two or more species in the same system (New and Zimmermann, 2010). It is considered a strategy potentially more sustainable than monoculture due to the reutilization of waste products of one species by another (Chopin and Yarish, 1999). The main objective of polyculture is to take advantage of the food existing in the system without that species may compete for resources (Cohen & Ra'anan,1983; Wohlfarth et al., 1985). This, generally, it is cultivated two or more species with different feeding habits and spatial habits, characterizing a multispatial and multitrophic aquaculture system (MMAS). Therefore, the choice of the organisms to produce is very important.

It is known that there are many advantages growing tilapia with prawns (Uddin et al., 2006; Bessa-Junior et al., 2010). The Nile tilapia has husbandry characteristics that make it suitable for cultivation with prawns. In this case, only the fish are fed, whereas the prawns feed on leftover food, faeces, and nutrients deposited on the bottom of ponds (Santos and Valenti, 2002). So MMAS increases productivity at a low cost and without additional environmental impact (New, 2000), promoting a better use of space, water, and facilities. Nevertheless, it is possible to optimize the use of resources and increase the efficiency of the systems to minimize negative impacts.

Also, polyculture practice can be combined with substrates that may improve the efficiency of aquaculture systems (Uddin et al., 2009). Substrates are colonized by periphyton, which can be used as food for aquatic organisms and promote nutrient cycling. It also allows prawns to explore vertical dimensions in the ponds, increasing useful area for benthic species. Thus, it reduces agonistic encounters and social interactions, improving the welfare of the animals allowing culture intensification (Tidwell et al., 1999, 2000 and 2001). Therefore, polyculture with the addition of substrates might result in benefits to production. Tilapias and prawns are able to re-suspend sediments (Ritvo et al., 2004; Kimpara et al., 2011), exposing organic matter to aerobic conditions favoring oxidation and recovering of nutrients to water column. Therefore, in ponds with substrates, part of the re-suspended material will be available for periphyton communities. Some studies showed that Nile tilapia grows better ingesting periphyton than filtering algae suspended in the water column (Hem and Avit, 1994; Guiral et al., 1995; Huchette et al., 2000; Azim et al., 2003). Thus, addition of substrate may improve growth and production for both prawns and tilapias (Uddin et al., 2006). Moreover, the use of substrates brings benefits to farmers that live in poor regions with restrict access to resources, as commercial diets or alternative feed materials (Nandeesha, 2003).

The development of sustainable aquaculture should include economic, environmental and social dimensions (Pillay, 1992). They are inseparable and essentials to establish a perennial activity. But the greatest challenge is to measure the sustainability (Boyd et al., 2007). It is essential to assess the efficacy of sustainable practices. Currently, some indicators were developed to assess sustainability in aquaculture (EAS, 2005; CONSENSUS, 2005; Boyd et al., 2007; EVAD, 2008; Valenti, 2008; Valenti et al., 2011). Nonetheless, there are few works using sustainability indicators to assess aquaculture experiments until now. Thus, this work used this tool to evaluate the environmental sustainability of the polyculture of prawn and Nile tilapia with two types of substrate, one composed by bamboo and the other by geotextile mantle. Both substrates are low cost and easy to obtain. Bamboo is a natural substrate and can be taken straight from the bush in neotropical regions once they are non-native and invasive plants into the Atlantic rain forest (Matos & Pivello 2009). Geotextile mantle has a large surface area and high porosity, allowing more area and habitat diversity for fixing periphyton. The present research aimed to provide information on the environment aspects of MMAS systems through environmental indicators, which can contribute to sustainable development of the aquaculture production. This work is part of a larger project that includes the evaluation of periphyton growth, population structure of prawns, economic analyses, nutrient balance, animal food preferences and zooplankton.

2. Materials and Methods

2.1. Experimental site and design

A 5-month experiment (December 2011 to May 2012) was conducted in the Crustacean Sector at São Paulo State University (CAUNESP), Jaboticabal, São Paulo, Brazil (21°15’22’’S, 48°18’48’’W). Twelve earthen ponds with 0.01 ha and 1 m of water depth were used. It was used three treatments in a completely randomized design: (1) control, without substrate (WS); (2) geotextile mantle substrate (GS); and (3) bamboo substrate (BS). Each treatment had four replicates.

2.2. Pond preparation, stock and management

The ponds were drained and exposed to sun until became completely dry. All remained aquatic vegetation and sediment were removed. Then, they were limed (250 kg ha^{-2} $CaCO_3$). After 3 days, the ponds were filled with water from a reservoir after passing through a mechanic filtering system. Pond inflow water was adjusted to compensate water losses by seepage and evaporation. The substrates were arranged vertically with the aid of a float attached to air bottles. On the day after, the ponds were fertilized with urea and simple superphosphate at the rate of 2 kg N ha^{-1} and 8 kg P_2O_5 ha^{-1}, respectively. Then, ponds were left 10 days to allow plankton development in the water column and periphyton growth on substrates. After this period, all ponds were stocked with Amazon river prawns (*Macrobrachium amazonicum*) at 22 individuals m^{-2}. Nile tilapias (*Oreochromis niloticus*) were stocked at 1.1 individuals m^{-2} after two weeks, allowing prawns adaptation. The initial mean weight of prawns and tilapias were 0.03 ± 0.01 g and 29 ± 1.13 g, respectively.

The same feed regime was maintained in all ponds. The prawns were fed with pelleted diet (35% crude protein) daily at a rate of 10% of body weight two times a day during the first 2 weeks, before tilapias stocking. Tilapias were fed daily with a pelletized commercial diet (40% crude protein in the first month and 28% for the rest of the culture period) at a rate of 5-1 % of tilapia biomass, according to development phase. Feed was divided into two equal portions and distributed at 12:00 and 16:00 hours every day. The animals were randomly sampled in monthly intervals to measure the length and weighing of 30 tilapias and 50 prawns individually to check their growth and to calculate feed requirement. Emergency aerators were used when dissolved oxygen declined bellow 1.5 mg L^{-1}.

2.3. Determination of water quality parameters

Water quality was monitored periodically through samples analyzed in laboratory and *in situ* measurements, at approximately 30 cm depth (Tables 1 and 2).

Table 1. Water variables and methods of analysis.

Variable	**Method of analysis/apparatus**	**Sample frequency/time of sampling**	**Reference**	**Apparatus specification**
Temperature	Thermistor (digital oxygen meter)	Daily (7h30/16h00)		YSI (Yellow Springs, OH, USA)
Transparency	Secchi disc	Biweekly (16h00)	Boyd (1979)	
Total Suspended Solids	Total Solids Dried at 103-105^{o}C	Biweekly (08h00)	APHA (2005)	
pH	Digital pH meter	Weekly (07h30)		YSI Professional plus model
Dissolved Oxygen	Digital oxygen meter	Daily (7h30/16h00)		YSI Professional plus model
N-total amonia	Phenate, colorimetric	Biweekly (08h00)	APHA (2005)	
N-nitrite	Colorimetric	Biweekly (08h00)	APHA (2005)	
N-nitrate	Cadmium reduction	Biweekly (08h00)	APHA (2005)	
N-total	Persulfate, cadmium reduction	Biweekly (08h00)	APHA (2005)	
P-soluble orthophosphate	Stanous cloride, colorimetric	Biweekly (08h00)	APHA (2005)	

Table 2. Averages (± SD) and range if variations (in brackets) of water quality parameters during the experiment for the systems: Without Substrate (WS), Geotextile Substrate (GS) and Bamboo Substrate (BS).

Water quality parameters	**Period**	**Treatments**[a]		
		WS	**GS**	**BS**
D.O. (mg/L)	Morning	4.50 ± 1.34 (0.80-9.38)	4.03 ± 1.54 (0.77-9.15)	4.07 ± 1.24 (0.79-8.99)
	Evening	11.42 ± 0.33 (2.63-17.35)	10.40 ± 0.42 (2.56-17.35)	10.91 ± 0.35 (2.64-18.73)
pH	Morning	7.87 ± 0.45 (7.18-9.13)	7.88 ± 0.15 (7.21-8.79)	7.71 ± 0.20 (7.14-8.25)
	Evening	9.28 ± 0.10 (8.06-9.78)	9.10 ± 0.11 (7.58-9.50)	9.23 ± 0.60 (7.68-9.56)
T (ºC)	Morning	27.1 ± 0.90 (20.5-29.4)	27.1 ± 0.88 (20.5-29.3)	27.1 ± 0.9 (22.7-29.5)
	Evening	30.18 ± 1.05 (24.1-33.3)	29.99 ± 0.98 (24.1-33.0)	30.1 ± 1.0 (24.7-33.8)
N-total ammonia (µg/L)	-	138 ± 35 (17-561)	143 ± 30 (26-465)	109 ± 24 (7-304)
N-nitrite (µg/L)	-	8.1 ± 3.6 (0.2-69.4)	11.2 ± 3.2 (0.6-70.7)	5.8 ± 1.6 (0.4-21.1)
N-nitrate (µg/L)	-	53 ± 27 (1.8-242)	85.6 ± 25.3 (1.5-270)	43.2 ± 24.5 (1.4-169)
Transparency (cm)	-	34.6 ± 2.11 (8-74)	39.5 ± 4.61 (13-82)	35 ± 4.68 (13-74)

[a] No significant difference was found among treatments, by ANOVA, F test (p>0.05)

2.4. Sediment quantification and nutrient analyzes

Tripton samplers were used to evaluate sediment deposition. These were made with six 1.876 L PVC tubes, with 9.7 cm diameter and 25.4 cm long (Figure 1). One tripton sampler was placed inside each pond. They were set up in the pond bottom 48 h in biweekly intervals. Samples were dried at 95-100^{o}C as described in AOAC (1995, method 934.01) and analyzed in laboratory to determine the contents of nitrogen, phosphorus and energy. The nutrient content measured were: nitrogen (method: 4500-Norg C. Semi-Micro-Kjeldahl, APHA 2005), phosphorus (method: metavanate colorimetry, applied to samples previously incinerated in a muffle furnace for 4 hours at 550 ^{0}C, Michelsen, 1957), energy (method: calorimeter bomb, IKA C2000 basic) and carbon (method: 920.153, AOAC 1995).

Figure 1. Tripton samplers.

2.5. Estimation of greenhouse effect gases

Dioxide carbon and methane emissions/absorption were estimated by samples obtained during the day (13:00 h) and at night (21:00 h) monthly. Two types of gas emission were analyzed: diffusive and bubbling. For the first, the diffusion at the air-water interface was evaluated by the balance method. A diffusion chamber, contained air inside collected as close as possible to the interface air-water is placed in contact with the surface of the water. Thus, the diffusion of gas from/to the water and the air inside the chamber starts to create a partial equilibrium of the gas dissolved in the water and inside the chamber. Samples of air inside the chamber were collected in periods of 1, 2 and 4 minutes to determine the flow of gas. Then, those air portions (samples) were placed in transfer tubes for analysis by gas chromatography (Construmaq, St. Carlos-SP). For the bubbling emission, the amount of CO_2 (at high concentrations) was determined using a gas chromatograph with TCD detector (Thermal Conductivity

Detector) and CH_4 and CO_2 (at low concentrations) with FID detector (Flame Ionization Detector). The methods are described in Matvienko et al. (2001).

Data on daily photoperiod were obtained from the agrometeorological station of UNESP Jaboticabal. These values were used to weight the hours of sunshine and hours of darkness in emissions. The final value was the sum of emissions daytime and nighttime throughout the experiment. The mass of gases was calculated using the emission and absorbed mass of CO_2 + CH_4 (measured in equivalents of CO_2) according to IPCC guidelines (IPCC, 2006).

2.6. Harvesting, estimation of yield parameters and nutrient analyzes

At the end of the experiment, all fish and prawn were collected. Each pond had 10% of prawns randomly sampled and all fish analyzed. They were weighted (Precision Balance Marte-AS2000C; 0.1 g precision) and measured (wood caliper; 1 mm precision). The weight gain per fish and prawn was calculated by deducing the average initial weight from the average final weight. To determine nutrient assimilation, 10 prawns and 3 tilapias were taken from each pond and analyzed according the methods described in the section 2.4. The production was calculated for 1 hectare and the values found were 5.23 ± 0.20 t ha^{-1} (WS), 5.47 ± 0.44 (GS) and 5.33 ± 0.48 (WS).

2.7. Statistical Analyses

The mean values obtained of water, sediment and gases were analyzed using SAS software (version 9.1). Data were subjected to normality and homogeneity of variances analysis using the Cramer-von Mises and Brown-Forsythe tests, followed by analysis of variance (ANOVA). When significant results were obtained, means were compared using Student-Newman Keuls test.

2.8. Environmental sustainability indicators

Environmental sustainability was measured according to Boyd et al. (2007), Valenti (2008) and Valenti et al. (2011), based on four aspects: (A) the use of natural resources; (B) efficiency in the use of resources; (C) pollutants released to environment (D) the conservation of genetic diversity and biodiversity (Table 3).

A. The indicators used to measure the use of natural resources were:

1. Space: used area per production. The used area is the sum of pond areas, monks and channels for water.

2. Water: Total volume of water used per production. The total volume was obtained for each pond by the sum of the volume to fill it with the reposition water volume. Then it was made the average of each treatment ponds.

3. Energy: Measures the total energy applied to the system in its different types. Were considered as energy applied, the post-larvae (cal g^{-1}), feed (cal g^{-1}), electricity consumption (kW h^{-1}) and human labor. In the latter, it was considered a total of 489 hours of work for control (WS) and geotextile substrate (GS) and 1025 hours for bamboo substrate (BS). The difference is due the work to harvest and elaborate the bamboo substrate. Energy expenditure per hours for human work in agriculture is about 500 kcal (Mello, 1986). All units were converted to megajoules (MJ).

4. Material: Mass of nutrient applied in the system per production.

4.1. Nitrogen: The mass of nitrogen applied is the sum of nitrogen contained in the feed offered, fertilizer used in the ponds and animals stocked.

4.2. Phosphorus: The mass of phosphorus applied is the amount of phosphorus contained in the feed offered added to phosphorus fertilization used in ponds and animals stocked.

4.3. Carbon: The mass of carbon applied is the amount of carbon contained in the feed offered and animals stocked.

B. The indicators used to measure the efficiency in the use of resources were:

5. Materials: Mass of material applied per mass of the same material incorporated in production.

5.1. Nitrogen: The mass of nitrogen applied is the sum of nitrogen contained in the feed offered, in the fertilizer used in the ponds and in animals.

5.2. Phosphorus: The mass of phosphorus applied is the sum of phosphorus contained in the feed supplied, the phosphorus in fertilization used in ponds and in animals.

5.2. Energy: The energy recovered in production is the sum of the average energy content of animals in each treatment multiplied by production. The energy applied is described in item 3.

C. The indicators used to measure pollutants released to the environment and accumulated inside ponds were:

6. Load of nitrogen: The mass of nitrogen released in effluent was calculated using the concentration of total nitrogen contained in wastewater multiplied by the volume of effluent released.

7. Load of phosphorus: The mass of phosphorus released in effluent was calculated using the concentration of total phosphorus contained in wastewater multiplied by the volume of effluent released.

8. Load of total suspended solids (TSS): the mass of total suspended solids was calculated using the amount of TSS contained in wastewater multiplied by the volume of effluent released.

9. Load of nitrogen accumulated in sediments: amount of nitrogen in the sediment generated per production.

10. Load of P accumulated in sediments: amount of phosphorus in the sediment generated per production.

11. Load of carbon accumulated in sediments: amount of carbon in the sediment generated per production.

12. Load of greenhouse effect gases: sum of the mass of gases released to the atmosphere and absorbed by ponds per production. The mass of gases was calculated using the emission and absorbed mass of CO_2 + CH_4 (measured in equivalents of CO_2) according to IPCC guidelines (IPCC, 2006).

Table 3. Environmental indicators and the formula used for the calculations.

Indicator	Formula
A - Use of natural resources	
Space	Area used (ha)/production (t)
Volume of water	Consumed volume (m^3)/production (t)
Energy applied	Energy used (MJ)/production (t)
Mass of nutrients	Material applied (kg)/production (t)
B - Efficiency in the use of resources	
Nitrogen Phosphorus	[mass recovered (kg)/mass applied (kg)] * 100 (%)
Energy	[recovered E (MJ)/E applied (MJ)] *100 (%)
C - Pollutants released and accumulated inside ponds	
Nitrogen Phosphorus Total suspended solids (TSS)	Mass released in the effluents (kg)/production (t)
Nitrogen Phosphorus Carbon	Mass accumulated in sediments (kg)/production (t)
CO_2 + CH_4	Mass of greenhouse effect gases (kg)/production (t)

D. The indicator used to measure the conservation of genetic diversity and biodiversity was the risk of cultivated species: scores = {1, 2, 3, 4, 5, 6 or 8}

1 = Local breed in open or closed system;

2 = Species from the same watershed in closed system;

3 = Species from the same watershed in open system;

4 = Allochthonous species or local species with reduced genetic variability or hybrid in a closed system;

5 = Allochthonous species or local species with reduced genetic variability or hybrid in an open system;

6 = Transgenic variety of any kind of species in a closed system;

8 = Transgenic variety of any kind of species in an open system.

2.9. Sustainability Index

Each indicator was converted into a performance scale, with scores ranging from 0 to 1. The treatment with the best indicator value (more sustainable when compared to the others) was arbitrary scored as 1, and the others were determined by proportion. Thus, the indicators were grouped into 5 categories: (a) Use of resources; (b) Efficiency of resources; (c) Water pollutants; (d) Sediment pollutants; (e) Greenhouse gas emissions. A sustainability sub-index was computed for each category by the average of their respective indicators. Only the category (e) represents the value of its unique indicator. The Sustainability Index was determined by the average of the 5 sub-indicators.

3. RESULTS

3.1. Usage and quality of water

There was no statistical difference among the treatments on the water usage and the nutrients accumulated in water (Table 4). All treatments showed a trend of nutrient and suspended solids accumulation (Figures 2).

Table 4. Volume of water used during the experiment and characteristics of the effluent for the following systems: Without Substrate (WS), Geotextile Substrate (GS) and Bamboo Substrate (BS).

Water	**Treatments**[a]				
	WS	**GS**	**BS**	**C.V. (%)**	**P**
Pond capacity (m³)	149.6	152.3	148.8	-	-
Total volume used (m³)	598	663	693	-	-
Reposition (% day^{-1})	2.11 ± 0.31	2.62 ± 0.49	2.59 ± 0.50	17.66	0.257
Effluent					
Nitrogen (mg L^{-1})	2.65 ± 0.58	3.36 ± 0.83	2.62 ± 0.29	21.12	0.209
Phosphorus (mg L^{-1})	2.19 ± 0.53	1.59 ± 0.51	1.60 ± 0.52	28.95	0.229
Carbon (mg L^{-1})	181.5 ± 84.9	132.6 ± 42.8	149.9 ± 35.1	37.82	0.574
Total suspended solids (mgL^{-1})	66.5 ± 58.2	81.4 ± 40.6	144.9 ± 60.9	55.34	0.148

[a]No significant difference was found among treatments.

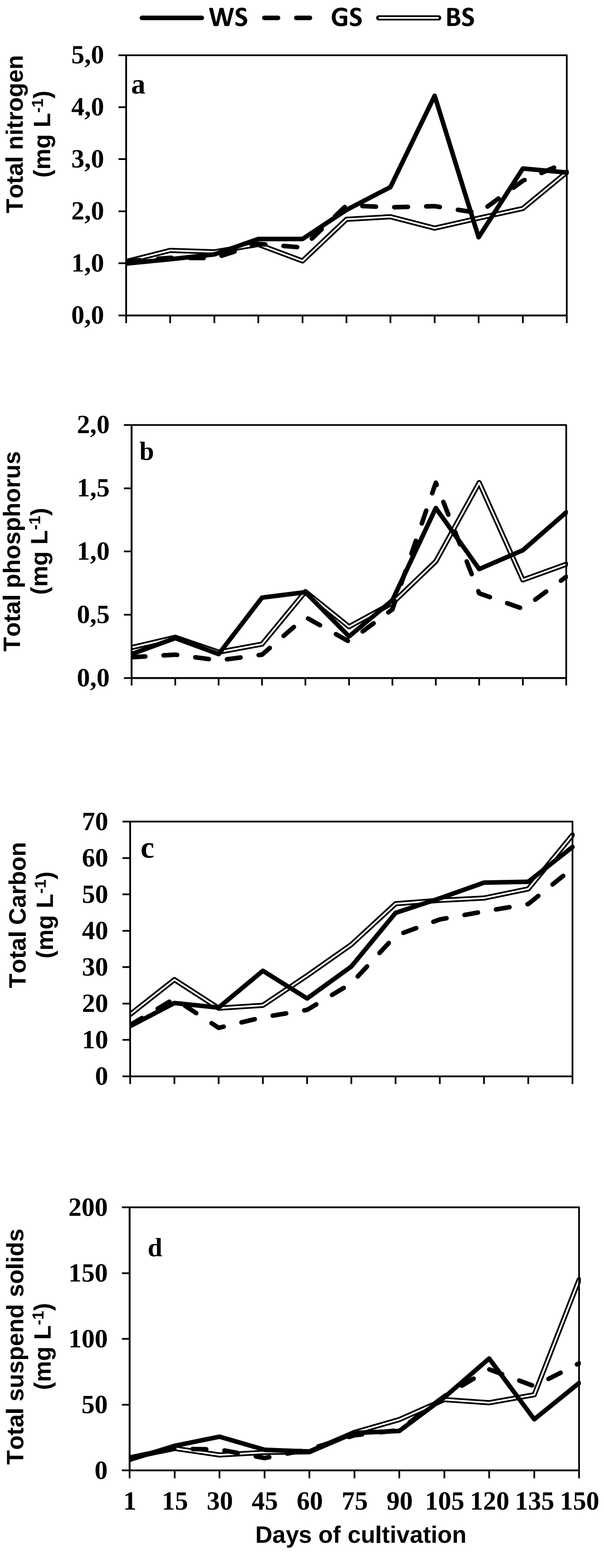

Figure 2. Concentrations of (a) nitrogen, (b) phosphorus, (c) carbon and (d) suspended solids during the culture.

3.2. Sediment

There was no statistical difference among the treatments on the sediment generation and the nutrients accumulated in the sediment (Table 5).

Table 5. Total sediment produced and the total amount of nutrients accumulated at the end of the experiment for the systems Without Substrate (WS), Geotextile Substrate (GS) and Bamboo Substrate (BS).

	Treatment				
Sediment	**WS**	**GS**	**BS**	**CV (%)**	**P**
Total produced (t ha^{-1})	5.51 ± 1.14	6.75 ± 1.85	5.66 ± 2.00	-	-
Total N in sediment (kg ha^{-1})	10.2 ± 5.0	12.6 ± 3.5	9.5 ± 3.6	20.75	0.230
Total P in sediment (kg ha^{-1})	5.81 ± 0.96	4.77 ± 0.76	4.07 ± 1.16	21.80	0.076
Total C in sediment (kg ha^{-1})	1270 ± 208	1301 ± 300	1100 ± 361	21.44	0.574

3.3. Gases

All treatments emitted methane, more during daytime and less during nighttime. Carbon dioxide was emitted only by GS during daytime while it was absorbed in all other treatments and periods (Table 6). Diffusive analyzes showed that all treatments maintained a pattern of absorption of greenhouse gases (Table 7). Bubbling emissions maintained a standard emission for all treatments. The WS treatment generated a negative balance (absorbed gases) and treatments with substrate had positive balances (emitted gases) (Table 7).

Table 6 – Total diffusive emissions of methane (CH_4) and carbon dioxide (CO_2) during the day and night for the following systems: Without Substrate (WS), Geotextile Substrate (GS) and Bamboo Substrate (BS).

Treatment	Period			
	Day		Night	
	CH_4 (kg ha^{-1})	CO_2 (kg ha^{-1})	CH_4 (kg ha^{-1})	CO_2 (kg ha^{-1})
WS	41.7 ± 50.4^a	-1311 ± 848^b	6.89 ± 29.1^a	-765 ± 364^a
GS	68.1 ± 38.4^a	190 ± 564^a	47.9 ± 22.6^a	-974 ± 662^a
BS	69.9 ± 45.4^a	-937 ± 47^b	37.3 ± 19.6^a	-178 ± 592^a
P	0.628	0.024	0.089	0.155

Mean values followed by different lower case superscript letters in the same row differ statistically ($P < 0.05$).

Table 7 – Diffusive and bubbling emissions of greenhouse effect gases for the following systems: Without Substrate (WS), Geotextile Substrate (GS) and Bamboo Substrate (BS). The balance is the sum of diffusive and bubbling emissions.

Treatment[a]	Difusive (kg ha^{-1})	Bubbling (kg ha^{-1})	Balance (kg ha^{-1})
WS	-2027 ± 770	768 ± 436	-1259 ± 870
GS	-668 ± 994	1283 ± 831	615 ± 907
BS	-1007 ± 808	1270 ± 962	263 ± 305
P	0.134	0.187	0.121

3.4. Indicators

A- Use of Natural resources

The demand of space indicator was nearly the same for all treatments (Table 8). WS treatment used less water to produce 1 t but required more inputs of nitrogen and phosphorus than GS and BS. The energy needed in BS was higher than the other treatments.

B- Efficiency in the use of natural resources

GS treatment has the highest energy efficiency but the lowest nitrogen and phosphorus efficiency. Energy efficiency in BS was the lowest. However it was the most effective treatment for phosphorus (Table 8).

C- Pollutants released to environment and accumulated inside ponds

The load of nutrients released by the effluents to produce 1 t changed according to each treatment. For example, WS released more phosphorus, GS more nitrogen and BS more suspended solids. The treatment WS was the only which absorbed greenhouse gases while GS emitted more (Table 8). In sediment, BS had the lowest rates of nitrogen, phosphorus and carbon. GS had the highest amount of carbon (Table 8).

Table 8. Environmental sustainability indicators for the systems Without Substrate (WS), Geotextile Substrate (GS) and Bamboo Substrate (BS).

Item	WS	GS	BS
A - Use of natural resources			
Space (ha t-1)	0.22 ± 0.02	0.21 ± 0.04	0.22 ± 0.04
Water (m³ t-1)	6.814 ± 322	7.424 ± 964	7.410 ± 142
Energy (MJ t-1)	66212 ± 2443	62900 ± 2567	71264 ± 2295
Material (kg ha^{-1})			
Nitrogen	83.2 ± 7.2	77.7 ± 12.7	80.6 ± 11.7
Phosphorus	18.6 ± 1.4	17.7 ± 0.8	17.7 ± 0.6
Carbon	914 ± 82	861 ± 31	859 ± 25
B – Indicators used to measure the efficiency in the use of resources			
Material (%)			
Nitrogen	26 ± 0.4	26 ± 1.7	29 ± 2.3
Phosphorus	29 ± 0.5	27 ± 1.1	35 ± 2.2
Energy	39 ± 0.6	41 ± 2.9	35 ± 1.4
C - Pollutants released or accumulated in ponds			
Load in water (kg t-1)			
Nitrogen	5.72 ± 1.52	6.92 ± 1.43	5.28 ± 0.82
Phosphorus	4.76 ± 1.34	3.28 ± 1.01	3.27 ± 1.27
TSS	80.0 ± 13.5	124.0 ± 9.4	247.2 ± 100.5
Load in sediment (kg t^{-1})			
Nitrogen	1.95 ± 0.99	2.26 ± 0.70	1.82 ± 0.82
Phosphorus	1.11 ± 0.21	0.85 ± 0.07	0.76 ± 0.22
Carbon	244.7 ± 46.7	229.9 ± 36.0	205.2 ± 63.8
Load of greenhouse gases (kg t^{-1})	-241 ± 173	117 ± 171	51.5 ± 57.1

The conservation of genetic diversity and biodiversity indicator received the value 4 for all the treatments - Allochthonous species or local species with reduced genetic variability or hybrid in a closed system.

3.5. Environmental Sustainability index

There were little variations on the category use of resources, but GS has a slightly better score. For the efficiency of resources, BS treatment was more efficient and GS the least. The category “water pollutant” is favorable for WS and uncongenial for BS. “Sediment pollutant” sub-index that BS greatly improves the quality in sediment in comparison to the others (Figure 4). Gas emission sub-index is the category most disparate because WS treatment is far better than the others treatments (Table 9).

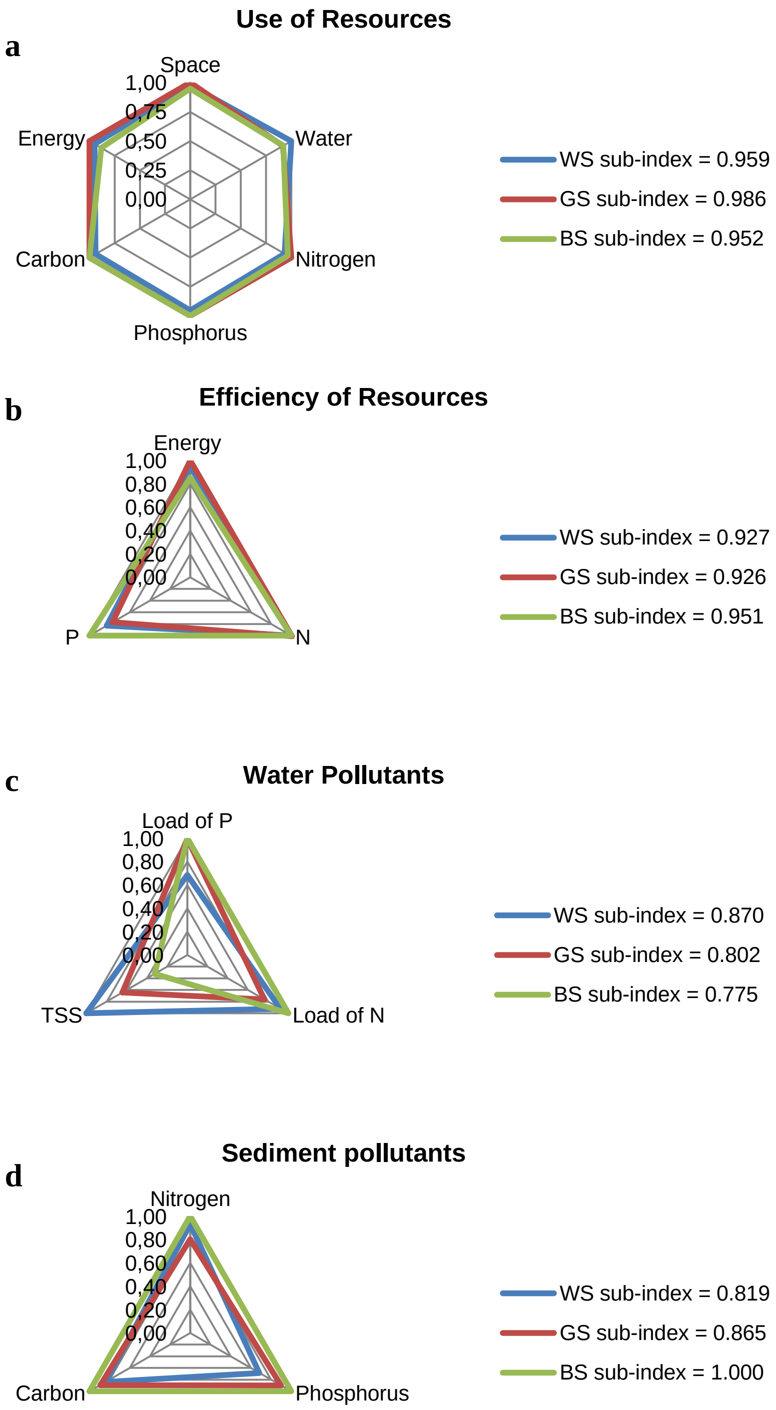

Figure 4. Indicators of sustainability obtained for each category (a) Use of resources; (b) Efficiency of resources; (c) Water pollutants; (d) Sediment Pollutants. The respective sub-index for each treatment are showed beside the graphic.

There were little variations among the index in general. The largest differences were observed in Sediment Pollutants and Gas Emission Index (Table 9 and figure 5). According to the Final Score, WS is the most sustainable treatment.

Table 9. Environmental Sustainability Index for each treatment. The final score is the average of all sub-indices.

Sub-index	Without Substrate	Geotextile Substrate	Bamboo Substrate
Use of resources	0,959	0,986	0,952
Efficiency	0,927	0,926	0,951
Water Pollutants	0,870	0,802	0,775
Sediment Pollutants	0,819	0,865	1,000
Gas emission	1,000	0,003	0,003
Biodiversity	1,000	1,000	1,000
Final Score	**0,929**	**0,764**	**0,780**

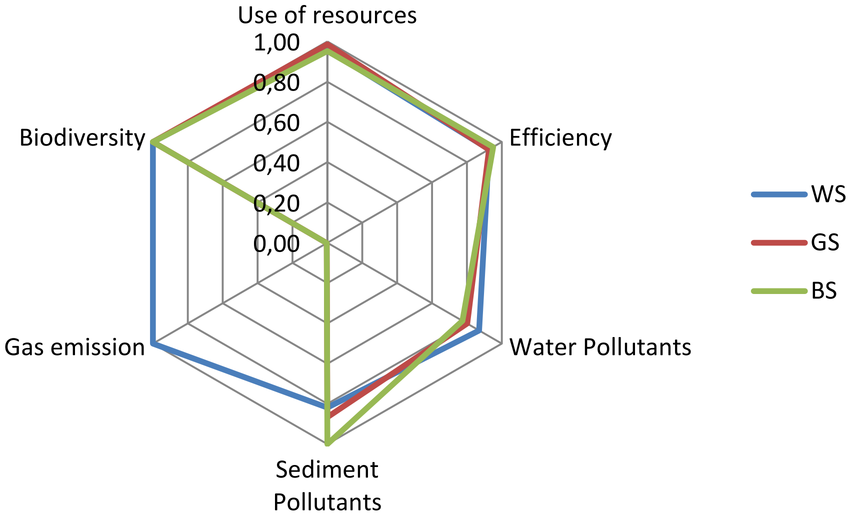

Figure 5. Environmental sustainability index.

4. DISCUSSION

4.1. Usage and water quality, sediment and gases.

Mean exchange rate was similar in all treatments (no statistical difference), which means that water demand was not influenced by substrates. The little variation occurred due to different pond infiltrations. Moreover, the daily water addition rate ranging 2.11 to 2.62% indicates an adequate hydric management since the expected loss by seepage and evaporation in ponds is expected to be nearly 9.5% day^{-1} (Verdegem et al., 2006). The nutrient accumulation in water for all treatments was expected since the systems were maintained without water renewal. Therefore, the effluent generated had high concentration levels of phosphorus and total suspended solids (TSS) which were higher than the target levels (0.3 and 50 mgL^{-1} for phosphorus and TSS, respectively) proposed by Global Aquaculture Alliance, a non-governmental organization which have made effluent standards for aquaculture (Boyd, 2003).

Aquaculture inputs (feed, fertilization, others) contributes to accelerate sediment generation. The development organic-rich sediments in the MMAS system can be a concern because the cycling of nutrients and other elements is rapid, and the efflux from these sediments to the water column is high (Holmer, 1992). But the addition of substrates did not increase the nutrient accumulation in sediment.

The increase of diffusive methane emissions by substrates treatments indicates that anaerobic decomposition should be occurred in substrates surface. Because of its low solubility and lack of ionic form, CH_4 might escape from the dissolved state in water into gas phase to form bubbles within the substrate that eventually escape to the atmosphere (Tokida et al., 2013). The greater absorption of CO_2 during daytime was expected because photosynthesis activity is normally higher than respiration. However, diffusive emission of CO_2 during daytime by GS treatment was not expected. Organic matter could be accumulated in geotextile substrate, which in contact with oxygen available in water column increase aerobic decomposition and the emission of CO_2. It is important to note that we did not measure the emissions of N_xO, which could affect the balance of greenhouse effect gases results.

4.2. Indicators

4.2.1. Use of resources

To understand the indicators is necessary to look at the numbers and try to interpret what is the reason for the value found and what have occurred in the system. The values of space indicator are nearly equal, which means that using this type of substrates did not affect the area demanded. In fact, the substrates do not decrease or increase the area because they were inserted into the pond and do not use an adjacent space. Moreover, substrates did increase the production as it was expected. The area used is adequate for the system (supplemental feed and no water exchange) utilized in this experiment which is expected to demands 0.125 – 0.500 hectare to produce 1 t in one year (Costa-Pierce at al., 2010).

The variations in water use indicator were most probably due to different seepage and evaporation of the ponds. There is no evidence that this may be related to the type of substrate. The nutrient demand indicators suggest that treatment WS demands more nutrient inputs. It makes sense, since the animals depends on feed offered, while in GS and BS animals have the possibility of consuming periphyton.

The energy used was higher in BS due to the effort (manpower and machines) to obtain and build the bamboo substrates. The GS treatment demanded less energy than WS because the energy expended to obtain the geotextile substrate was not computed, once it was bought at the marketing. So, the better value for GS is due to the higher productivity.

4.2.2. Efficiency in the use of resources

The indicator of energy efficiency was higher for GS and lower in BS as explained previously. Comparing to monoculture systems, intensive marine shrimp production have a nutrient efficiency of 7.8% and 22.9% for phosphorus and nitrogen, respectively (Boyd, 2007) and nursery freshwater prawn systems 5.7 % and 11.4% for phosphorus and nitrogen, respectively (Proença, 2012). Thus, the values obtained in the present work (26-29% for nitrogen and 27-35% for phosphorous) corroborate that MMAS system increase the efficiency of nutrient used. This result is because the existence of two species that consumes nutrients that would be wasted in monoculture.

4.2.3. Pollutants released and accumulated inside ponds

The absorption of gases was observed in ponds without substrate. It means that MMAS system may absorbed greenhouse gases, but the addition of substrate to this system may led to emission. Further research should be performed to confirm if the use of any kind of substrates increase the emission of greenhouse gases.

Each treatment generated a different nutrient accumulation in the effluent. BS released more TSS, WS more phosphorus and GS more nitrogen (Table 8). In this case an index seems to be more effective to compare treatments, because they combine all these indicators into a single value, which minimize the probability to obtain differences by chance.

4.2.4. Conservation of genetic diversity and biodiversity

Despite being a species of wide occurrence in Brazil, populations of *M. amazonicum* were captured in the Amazonia basin, which is far from where the experiment was conducted. Thus, it is considered allochthonous species. Tilapia was imported from other territory and so it is also an allochthonous species. The escape of these animals can lead to a negative interaction with wild populations through competition, transfer of diseases and pathogens, and interbreeding (Jensen et al., 2010). However, the risk decreases when the system is closed, because it has no water exchange and the possibility of escape of the animals is reduced. This indicator is site-specific and the result should be totally different if the culture would be performed in the aquatic basin of origin of the species used.

4.3. Environmental Sustainability Index

The Environmental Sustainability Index represents the summary or the aggregation indicators that allows the full comparison between the systems used. There are little differences between the three systems in the sub-index use of resources and efficiency. However, the systems with substrates (GS and BS) had better scores in sediment pollutants (especially BS, which had the best score) but these systems had worse water pollutants and gas emission sub-index scores. It means that using these substrates may improve MMAS sediment sustainability but at cost of water quality and the emission of gases. The most evident difference among treatments is the gas release/absorption. The maximum score given to WS is due to its absorption of greenhouse gases, while GS and BS emitted these gases. That sub-index was crucial to determine the final index score favorable to WS. That's the main reason to WS be considered the most sustainable system. Nevertheless, if we joint the 3 sub-indices gas emission, water and sediment in a single sub-index calculated to show the pollutants released to environment and accumulated inside ponds the result would be different. Instead of six we would have four sub-indices and the index score values calculated changed to: WS = 0.945; GS = 0.867; BS = 0.874. It means that WS would still the more sustainable, but the values of sustainability were improved for those treatments using substrate.

4.4. The use of indicators and indices

Indicators are expected to, be simple, quantifiable, sensitive to changes, show wide scope and allow timely identification of trends (Harger and Meyer, 1996). From a general to specific information, indicators and indices are a very objective tool to provide direct information to summarize the environmental sustainability of the system. The indicators used are single numbers that express several complex phenomena and detected changes. Moreover, not only the negative impacts were detected, but the positives as well (e.g. gas absorption instead of emission). It is important because it is difficult to measure positive impacts because monitoring protocols, performance measures and metrics are mostly designed to identify negative impacts (Chopin et al 2012).

Indicators provided single information of each important aspect of the environmental concern. They allow the identification of the weak and strong points evaluated for the system. The indicators converted into indices generating a final sustainability score, which may be useful to compare treatments in experiments (Valenti et al., 2011). The main constrain is to detected the real differences from those caused by chance. Statistical analysis to compare indicators among treatments can be used. Nevertheless, statistical tests are not efficient to detect small differences in parameters, which show large variability. Further studies should be performed to look for suitable tests. On the other hand, when we consolidate the indicators in indices or sub-indices, the mistakes may be minimized.

In this work, the indicators and indices were grouped into different categories to make easier the interpretation of the system function. The index allowed a holistic interpretation of the systems while the indicators provided specific information. Moreover, the information generated by indicators and indices can be used as a database for compare different aquaculture systems. The studies using indicators need to use international recognized methods to allow the comparison of different aquaculture systems.

5. CONCLUSIONS

The use of indicators and indices are adequate and effective to evaluate the environmental sustainability in aquaculture. The main aspects of the system were detected, identified and quantified. However, it is important to emphasize the subjectivity in the construction of the Index, which is dependent on the interpretation and choices of sub-indices of the researcher. Nevertheless, we can conclude that the system WS is the most environmentally sustainable. So, despite of the benefits that the use of substrates in aquaculture may promote, its use leads to a reduction in the sustainability of the system by release gases that may cause several impacts on global warming. The present research also provided information of the environmental sustainability of MMAS systems. Further researches should be made attempting to assess and compare differences on manipulating and re-combining the sub-indices to achieve the final sustainability score.

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VII. CONSIDERAÇÕES FINAIS E CONCLUSÃO

A utilização de indicadores e índices são adequados e eficazes para avaliar a sustentabilidade ambiental em aquicultura. Através dessa ferramente foi possível detectar e quantificar os principais aspectos do sistema. No entanto, é importante salientar a subjetividade na construção do índice, que é dependente da interpretação e escolhas de sub-índices do pesquisador. Contudo, podemos concluir que o sistema WS é o mais ambientalmente sustentável. Assim, apesar das vantagens na utilização de substratos na aquicultura, a sua utilização conduz à uma redução da sustentabilidade ambiental do sistema pela libertação de gases, que pode causar vários impactos de aquecimento global. A presente pesquisa também forneceu informações da sustentabilidade ambiental dos sistemas de MMAS. Futuras pesquisas devem ser feitos na tentativa de avaliar e comparar as diferenças na manipulação e re-combinar os sub-índices para alcançar a pontuação final sustentabilidade.