



Emission and absorption of greenhouse gases generated from marine shrimp production (*Litopenaeus vannamei*) in high salinity

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ABSTRACT

This study aimed at identifying and quantifying greenhouse gas fluxes (CH₄, CO₂, and N₂O) in *Litopenaeus vannamei* shrimp nurseries submitted to different culturing conditions. The experiment was carried out with a completely randomized design, with two treatments and four simultaneous replicates, totaling eight experimental units. Two management systems were tested. The first one (M1) used a stocking density of 92 shrimp/m² and fertilizer maintenance through the application of calcium nitrate and molasses. The second one (M2) used a stocking density of 14 shrimp/m² without fertilizer maintenance. Feeding in both treatments consisted in supplying ration through the volley method. The results showed that there were variations in the pattern of gas emission in both treatments and in the concentrations of the evaluated gases. The recorded mean values of total gas flux were −314.87 mg/m²/day of CH₄, −3773.51 mg/m²/day of CO₂, and 2.47 mg/m²/day of N₂O in M1; and 653.89 mg/m²/day of CH₄, 497.52 mg/m²/day of CO₂, and 25.59 mg/m²/day of N₂O in M2. The results obtained in this study suggest that environmental and management conditions interfere with the cultivation system, which acts as either a source or drainage of gases. These emissions from shrimp farming are potentially critical, mainly due to N₂O emissions, when compared to emissions from other production systems. Conversely, the cultivation of *L. vannamei*, particularly when carried out with the use of organic fertilizers such as molasses, presented a potential absorption of gases such as CH₄ and CO₂. The greatest fluxes of gases occurred at the beginning of the cultivation due to the initial fertilization. In addition, the contribution of molasses probably favored denitrification and increased natural productivity, which may have contributed to a lower emission of these gases compared to emissions of other systems where molasses was not used.

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1. Introduction

Aquatic ecosystems can be a significant source of emissions of greenhouse gases (Cole et al., 2007; Dean and Gorham, 1998; Enrich-Prast and Pinho, 2008; Silva et al., 2016). Most of the data that supports this information is based on studies carried out in rivers and hydroelectric reservoirs (Almeida et al., 2016; Bastviken et al., 2011; Cailleaud et al., 2014; Deshmukh, 2013; Faria et al., 2015; Marcelino et al., 2015; Sbrissia et al., 2011). Few studies have quantified the emission of gases generated by productive activities such as aquaculture (Boyd et al., 2010; Datta et al., 2009; Frei and Becker, 2005; Orjuela, 2011; Schott et al., 2016; Yang et al., 2012).

Considering anthropic actions, agricultural and farming activities are currently one of the main sources of greenhouse gas emissions to the atmosphere (IPCC, 2016; Kumar, 2013; Kumar et al., 2013; Osório and Azevedo, 2014). This is especially the outcome of changes in land use and increased confinement of crops in search of better production results (IPCC, 2016; Henriksson et al., 2017; MCTI, 2013). Agricultural and farming activities contribute to approximately 25%, 65%, and 90% of the total anthropogenic emissions of CO₂, CH₄, and N₂O, respectively (IPCC, 2016). Thus, food production alone accounts for about 50% of the global greenhouse gas emissions into the atmosphere (Goodland and Anhang, 2009; Johnson et al., 2005). These emissions present direct effects on global warming and consequently on changes in climatic patterns.

Although aquaculture is an activity that is influenced by climate change, being directly affected by the inconstancy and severity of these changes, it is also an activity that contributes to the greenhouse effect through the basic processes that occur in the soil-

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water-animal system; and it can act as either a source or drainage of these gases depending on the management practices that are adopted (Boyd et al., 2010; Cole et al., 2007; Enrich-Prast and Pinho, 2008; Orjuela, 2011; Santos et al., 2008; Schott et al., 2016; Yang et al., 2012). Aquaculture has stood out among the main systems of animal production in confinement, presenting itself as an efficient way to produce animal protein to feed the growing world population - mainly because it presents several advantages in relation to other activities such as short production cycles, high technological level, and small space demand for production (FAO, 2016; Samuel-Fitwi et al., 2012).

The impacts caused by aquatic organism production systems vary greatly according to the cultivated species and levels of production intensification (Ewoukem et al., 2012). There are systems considered less impactful which may even offer some environmental benefits (Godoy et al., 2018; Kimpara et al., 2010; Mok and Gaziulusoy, 2018; Moura et al., 2016; Valenti et al., 2011). About 16.6 million tons of carbons are submerged in aquaculture tanks annually; of this total, 13.1 million tons are in freshwater systems, and 3.5 are in brackish water cultivating systems. This amount is about half the quantity observed in natural lakes and inland waters, which together amount to 34 million tons (Boyd et al., 2010). In addition, if worldwide aquaculture continues to increase at the current pace, with an annual average growth of almost 8%, it could account for about 6% of the anthropogenic N₂O emissions by 2030 (Hu et al., 2012).

These facts suggest that the emission of greenhouse gases from aquaculture activities may be a problem, with major regional contributors influencing global climate change. Hence, knowing the emission dynamics of these gases is essential to evaluate aquaculture sustainability. The objective of this study was to identify and quantify the fluxes of greenhouse gases in the production of marine shrimp (*Litopenaeus vannamei*) under different cultivating conditions.

2. Material and methods

2.1. Study area

The study was took place in a commercial area of *Litopenaeus vannamei* shrimp farming, which had in ground excavated nurseries operated by the Aquarium Aquaculture farm (Lat. 5° 6' S, Long 37° 16' W), located in the Northeastern semi-arid region of Brazil (Fig. 1). According to the Koeppen classification, this region

presents a Bsh climatic type, with semiarid climate, low humidity, and low rainfall volume. The farm is located on the left bank of the Mossoró River estuary and built next to mangrove areas. It operates with a total water recirculation system, and comprises 800 ha of total area, with an area of 300 ha in operation containing about 80 fattening nurseries with sizes ranging from 0.26 to 15 ha.

2.2. Experimental design

The experiment was performed with a completely randomized design with two treatments. Each treatment was performed with four replicates, totaling eight experimental units and followed the same operational procedures adopted in the shrimp farm. Two treatments with different characteristics regarding the handling and initial stocking densities were defined. Management 1 (M1) consisted of four nurseries populated with a stocking density of 92 shrimp/m². This treatment received an initial fertilization of a mixture of 100 kg/ha of wheat bran, 30 kg/ha of calcium nitrate, 20 kg/ha of silicate, and 20 kg/ha of molasses; biweekly maintenance fertilizations were performed with the application of calcium nitrate in the proportion of 30 kg/ha along with a weekly application of molasses in the proportion of 10 kg/ha. Management 2 (M2) consisted of four nurseries populated with the density of 14 shrimp/m². This treatment received initial fertilization similar to that of M1 and no maintenance fertilization.

The supply of ration followed the volley method using three types of commercial feed with different compositions (Table 1). Phase 1 corresponded to the period between set up and 10 days of cultivation; Phase 2 corresponded to the period between 11 days of cultivation until shrimp reached 3 g in weight, and Phase 3 (fattening ration) corresponded to the period between the end of phase 2 and harvest.

Shrimp in both treatments were initially fed four times a day, being offered 10% of the biomass until the average weight of individuals reached about 1 g. The subsequent feed rate was gradually reduced to 2% of the biomass at the end of the experiment, with two feeding times per day. The total feed provided to the four nurseries of each treatment was 1967 kg for M1 and 4563 kg for M2, with mean values of 491.7 kg (±21.6) in M1 and 1140.7 kg (±174.3) in M2.

Samples were collected in both treatments at the beginning, middle and end of the cultivation (1st day: settlement, 26th day: Biometry, 53rd day: shrimp removal) in each experimental unit, totaling 53 days of cultivation. The emissions of diffusive and ebullient greenhouse gases were measured, identifying and quantifying emissions: methane (CH₄), carbon dioxide (CO₂), and nitrous oxide (N₂O). A number of independent variables were also measured at these time points to verify the influence of these parameters on the flux of gases - including temperature, salinity, dissolved oxygen, transparency, pH, total dissolved solids, total suspended solids, turbidity, electrical conductivity, total phosphorus, orthophosphate, and the concentrations of organic and inorganic carbon in the nurseries' water column.

The contribution of the surface diffusive emission in mg/m²/day was estimated using a diffusion chamber (Fig. A.1) (Santos et al.,



Fig. 1. Location the study area: Aquarium Aquicultura do Brasil shrimp farm in semiarid Northeastern Brazil.

Table 1
Characterization of the commercial rations used in culture.

Phases	CP (%)	P (%)	E.E. (%)	Granulometry (mm)
Phase 1	40	1.3	0.9	0.54 a 1.0
Phase 2	40	1.3	0.9	1.0 a 1.8
Phase 3	35	1.2	0.85	2.5

Subtitle: % CP: Percentage crude protein ratios; % P: Percentage of phosphorus ratios, % E.E: Percentage of ethereal extract.

2005). Sampling was conducted by positioning the diffusion chamber facing down on the water surface; so gases coming from the ponds gradually tended to concentrate in the trapped air inside the chamber. Gas samples were subsequently collected with 30 ml syringes at 0, 1, 2, and 4 min after the chamber was placed on the water surface; these samples were stored in gas chambers. The collection of diffusion samples was performed during the day and during the night. Ebullient emissions were estimated with the aid of submerged inverted funnels (Santos et al., 2005) with a diameter of 0.0707 m² (Fig. A.1), fixed just below the water surface, with a water-filled vial attached to the funnels' top. These funnels were positioned close to the bottom of the nurseries so that the bubbles of gases upon detachment rose to the top of the funnel, accumulating inside the reservoir. The funnels were in the water for 24 h; the accumulated gas was collected and stored in gasometric chambers. Subsequently, the gas chambers were transported to the laboratory to determine the concentrations of CH₄, CO₂, and N₂O through chromatography (chromatograph model Hplc Shimadzu). With the result of the chromatographic analysis the successive increase or reduction of the concentration of each gas in the chamber volume was determined. After that, the rate of increase (or decrease) of the gas mass contained in the chamber was calculated. These were measured percentages and then converted to ppm. The mean daily data for all gases obtained by diffusion and ebullience in each treatment were summed to obtain the total flux of each gas in mg/m²/day for the 24-h period; these results indicated whether the nurseries emitted or absorbed gases in each system.

Water samples were collected biweekly, twice a day, in the morning, and at night. The following water quality parameters were measured: dissolved oxygen (OD), temperature, pH, salinity, electrical conductivity, oxidation potential (ORP), turbidity, and total dissolved solids (TDS). Moreover, a multiparametric probe (Horiba U-50, Kyoto, Japan) was used in all experimental units. Samples were collected in the water column and conditioned for subsequent vacuum filtration in the laboratory using cellulose membrane filters (47 mm in diameter and 0.45 μm porosity) to measure chlorophyll *a*, ammonia, nitrite, nitrate, orthophosphate, total phosphorus, total carbon (TC), organic carbon (TOC), inorganic carbon (TIC) and organic matter (OM). The concentrations were determined by spectrophotometry according to recommendations specified for each analysis. At the end of the experiment, the measured parameters of evaluation of zootechnical performance were: final mean weight (g), apparent feed conversion factor (AFC), and survival rate (%). The water quality data and zootechnical parameters in both treatments are presented in Tables 2 and 3, respectively.

3. Results

The average values of the analyzed limnological variables were in the same range as those observed in other studies involving cultivation of *L. vannamei* (Lin and Chen, 2001, 2003; Gaona et al., 2011; Krummenauer et al., 2011; Santos et al., 2014; Jatobá et al., 2015) these values were considered suitable for the cultivation of marine shrimp.

3.1. Ebullient fluxes

The results obtained for the boiling fluxes showed that the bubbling emission values were always lower in the M1 treatment, which presented mean CH₄ emission values of 52.93 (±32.09) mg/m²/day. However, much higher values were found for M2, reaching an average emission of 340.37 (±499.12) mg/m²/day of CH₄. The same pattern can be observed for CO₂ emissions, which the average CO₂ fluxes in M1 and M2 were 20.71 (±26.66) and 41.65 (±61.54)

Table 2

Limnological characteristics of the water column of the nurseries of marine shrimp in the different treatments.

PARAMETERS	M1	M2	* Reference Levels
Salinity (ppt)	32.46	31.90	0.5–35
Transparency (cm)	31.56	33.75	30–50
Temperature (°C)	28.96	29.14	28–32
pH	8.43	8.50	6–9
ORP (mv)	121.36	137.89	400–500
Conductivity (us/cm)	45.98	45.92	23–71
Turbidity (NTU)	116.11	103.34	≤100
DO (mg/l)	7.56	7.31	5.0–9
% DO	119.16	116.54	–
TDS (g/L)	28.49	28.11	100
Chlorophyll A [ug/l]	11.90	5.69	≤30
Ammonia [ug/l]	162.37	178.00	100–1000
Nitrate [ug/l]	712.80	690.60	≤1000
Nitrite [ug/l]	4.38	2.68	≤10
Orthophosphate [ug/l]	217.94	99.78	5–200
TIC [mg/l]	27616.00	24360.00	–
TC [mg/l]	47228.81	44892.94	–
TOC [mg/l]	20532.00	19613.00	–
Total phosphorus [ug/l]	1212.74	505.12	1–100
OM (mg/L)	4.97	4.73	≤4
Suspended solids (mg/L)	551.00	212.00	500
Total solids (ml/L)	3.37	1.50	10

Subtitle: ORP: oxidation reduction potential, DO: Dissolved oxygen, TDS: total dissolved solids, TC: Total Carbon, TIC: Total Inorganic Carbon, TOC: Total Organic Carbon, OM: organic matter, M1: management systems 1, M2: management systems 2.

*These reference levels are the values considered ideal for shrimp development according to MPEDA (Marine Products and Export Development Authority), 1992.

Table 3

Zootechnical parameters for the two treatments.

PARAMETERS	M1	M2
Final average weigh (g)	6,32	9,4
Average daily gain (g)	0,12	1,18
Apparent feed conversion ratio	2,95	1,44
Biomass/há	651	332
Survival (%)	43	12
Cycles per year	4	6
Density (shrimp/m ²)	92	14

Subtitle: M1: management systems 1, M2: management systems 2.

mg/m²/day, respectively. For the ebullient emissions of N₂O, the emission mean values were close to zero in both treatments, with a reduced amplitude of variation, and mean fluxes of 0.25 × 10⁻² (±0.31 × 10⁻²) mg/m²/day in M1 and 0.3 × 10⁻² (±0.11 × 10⁻²) mg/

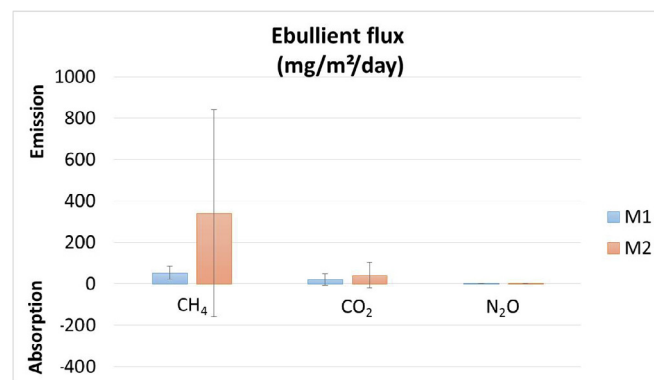


Fig. 2. Mean values and standard deviations of the ebullient flux (mg/m²/day) for each treatment. Subtitle: CH₄ – Methane, CO₂ – Carbon dioxide, N₂O – Nitrous oxide, M1: management systems 1, M2: management systems 2.

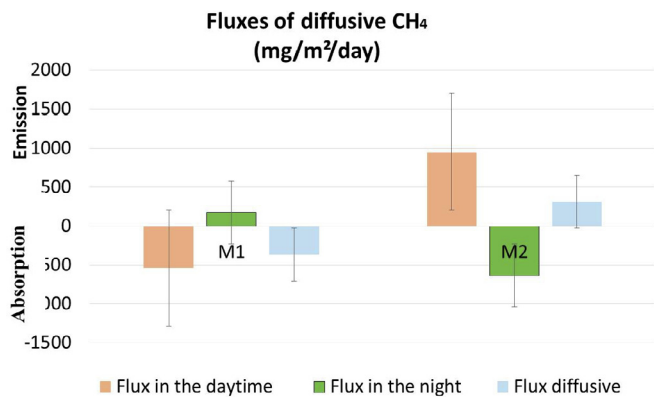


Fig. 3. Mean values and standard deviations of CH₄ diffusive flux (mg/m²/day) for each treatment. Subtitle: CH₄: methane, M1: management systems 1, M2: management systems 2, Absorption (negative values): gases that are absorbed by the system. Emission (positive values): gases released by the system.

m²/day in M2 (Fig. 2).

3.2. Diffusive fluxes

The CH₄ diffusive fluxes found showed that the diffusive emission rate in M1 presented negative flux in the daytime period with a mean value of -541.43 mg/m²/day and a positive flux at night with a mean value of 173.63 mg/m²/day. These results represented a CH₄ negative daily diffusive flux equivalent to -367.80 mg/m²/day. The inverse occurred in M2, which showed a high positive flux in the daytime period with a mean value of 951.5 mg/m²/day, and negative flux in the night period with a mean value of -637.97 mg/m²/day. These results represented a CH₄ daily diffusive positive flux with a mean value of 313.52 mg/m²/day (Fig. 3).

The CO₂ diffusive fluxes presented negative values in M1 and flux with positive values only in the nocturnal period, while the fluxes observed in M2 were always positive (Fig. 4).

In the verified CO₂ rates in M1, the daytime flux was high, with a negative mean value of -3797.9 mg/m²/day, while the nocturnal flux showed positive values with a mean value of 3.75 mg/m²/day, together representing a negative daily diffusive flux of -3794.22 mg/m²/day. The nurseries in the M2 treatment

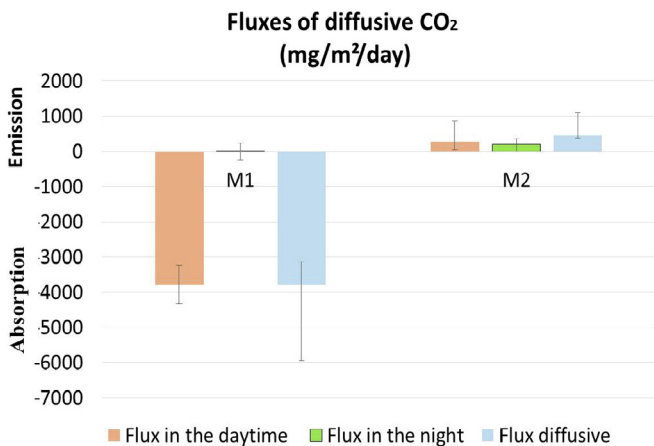


Fig. 4. Mean values and standard deviations of CO₂ diffusive flux (mg/m²/day) for each treatment. Subtitle: CO₂: Carbon dioxide, M1: management systems 1, M2: management systems 2, Absorption (negative values): gases that are absorbed by the system. Emission (positive values): gases released by the system.

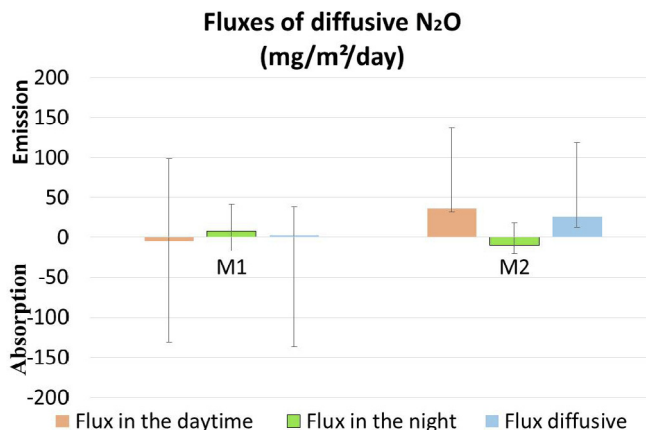


Fig. 5. Mean values and standard deviations of N₂O diffusive flux (mg/m²/day) for each treatment. Subtitle: N₂O: Nitrous oxide, M1: management systems 1, M2: management systems 2, Absorption (negative values): gases that are absorbed by the system. Emission (positive values): gases released by the system.

presented positive values in all observations, with a mean daytime flux of 264.45 mg/m²/day and a nocturnal flux of 191.40 mg/m²/day, representing a CO₂ daily diffusive flux of 455.86 mg/m²/day. The M2 nurseries presented only CO₂ emission throughout the study period, with no absorption by the system.

Even in small quantities, N₂O was emitted daily into the atmosphere in both treatments. In M1, the flux in the daytime period presented a mean value of -5.20 mg/m²/day while in the night period it presented a mean value of 7.67 mg/m²/day, representing a daily diffusive flux of 2.46 mg/m²/day. Conversely, the N₂O values observed in M2 were higher than those in M1, presenting flux mean values of 35.76 mg/m²/day in the daytime period and -10.17 mg/m²/day in the nighttime period, representing an N₂O daily diffusive flux of 25.58 mg/m²/day (Fig. 5).

3.3. The total flux of gases

The total flux of gas in mg/m²/day was obtained for each cultivating treatment from the mean values of the combined diffusive and ebullient fluxes. Variations in the standard emission fluxes of greenhouse gases were observed in the two treatments and in the

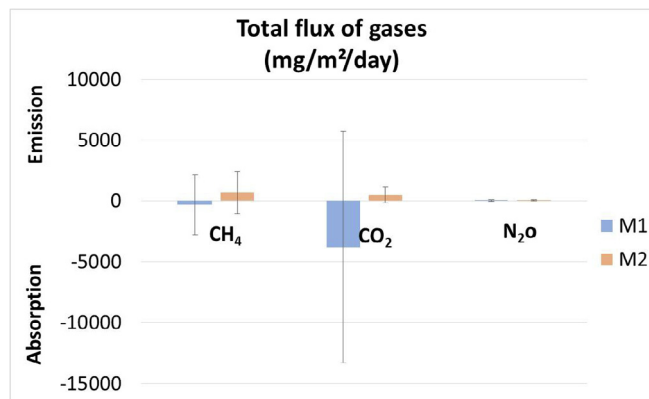


Fig. 6. Total flux (mg/m²/day) in marine shrimp fattening nurseries for the two treatments for all analyzed gases. Subtitle: M1: management systems 1, M2: management systems 2. Total flux: diffusive flux + ebullient flux. Absorption (negative values): gases that are absorbed by the system. Emission (positive values): gases released by the system.

concentrations of the evaluated gases. N₂O showed the lowest contribution in both treatments, while CH₄ and CO₂ showed different contribution patterns in M1 and M2, where the values of negative flux presented in M1 revealed the absorption of these gases in this treatment (Fig. 6).

Conventionally it is understood that positive (emission) fluxes occur when there is a transfer of gas from an aqueous to a gaseous medium, whereas negative fluxes (absorption) represents gas assimilation in the reverse direction, from a gaseous to an aqueous medium. The results of total fluxes obtained in M1 presented the following mean values and standard deviations: CH₄: -314.870 ± 2455.79 ; CO₂: -3773.511 ± 9524.75 ; and N₂O: 2.499 ± 92.42 ; while in M2 the values were: CH₄: 653.890 ± 1726.66 ; CO₂: 497.518 ± 644.06 ; and N₂O: 25.590 ± 65.45 .

High amplitude of variation in the fluxes of all gases throughout the study period was observed. Despite this pattern, M2 presented

higher values than M1 throughout the cultivation period. The CO₂ flux presented the highest values when compared to the other gases. Fig. 7 shows the monitoring of total CH₄, CO₂, and N₂O fluxes between the sampling periods in both treatments.

Out of the total gases produced, 55.7% corresponded to CO₂, 42.9% to CH₄, and 1.4% to N₂O (Fig. 8); the relative contribution of the diffusion process corresponded to the average of 93.4% of the total flux recorded for all gases produced by the nurseries, and the ebullient flux contributed only with 6.6% (Fig. 9). In addition to the results of the percentage distribution of gases in each treatment, different gas distribution patterns were observed in M1 and M2, where the two treatments presented values higher than 60% for different gases (Fig. 10). M1 presented 62.2% of CO₂, followed by 36.7% of CH₄, and 1.1% of N₂O, M2 presented similar values, but for different gases (64.3% of CH₄, 33.2% of CO₂, and 2.5% of N₂O).

4. Discussion

Variations were observed in the emission patterns of gases in two treatments used during the study period; similarly, variations were observed in the concentrations of the evaluated gases. Regardless of presenting a quantitatively lower contribution than the other gases, N₂O showed the same pattern in both treatments, being emitted in both M1 and M2. CH₄ and CO₂ showed different patterns in both treatments, with absorption in M1 and emission in M2. Thus, it is possible to relate that conditions similar to those exposed in M1 favor the higher CO₂ flux, while conditions similar to M2 favor the flux of CH₄. It should be remembered that the contribution of N₂O to greenhouse gas emissions was less than 5% for both treatments, being in the range described for tropical areas, that is, between 0 and 20% (IHA, 2010).

Yang et al. (2015) report that *L. vannamei* nurseries in South-eastern China acted as sources of greenhouse gases, emitting large amounts of CH₄ and CO₂ and little of N₂O. While the polyculture of carp with *L. vannamei* in the same region results in CO₂ absorption and emission of other gases. Hu et al. (2012) also state that aquaculture is a major contributor to greenhouse gas emissions. However, Boyd et al. (2010) mentions that aquaculture nurseries can, in addition to not emitting, act as gas drainage. Thus, there is no clear pattern related to how aquaculture activities can act because studies have reported opposite results, i.e, some report emission while others report absorption of greenhouse gases. Several factors may influence the quantity, type, and emission or absorption

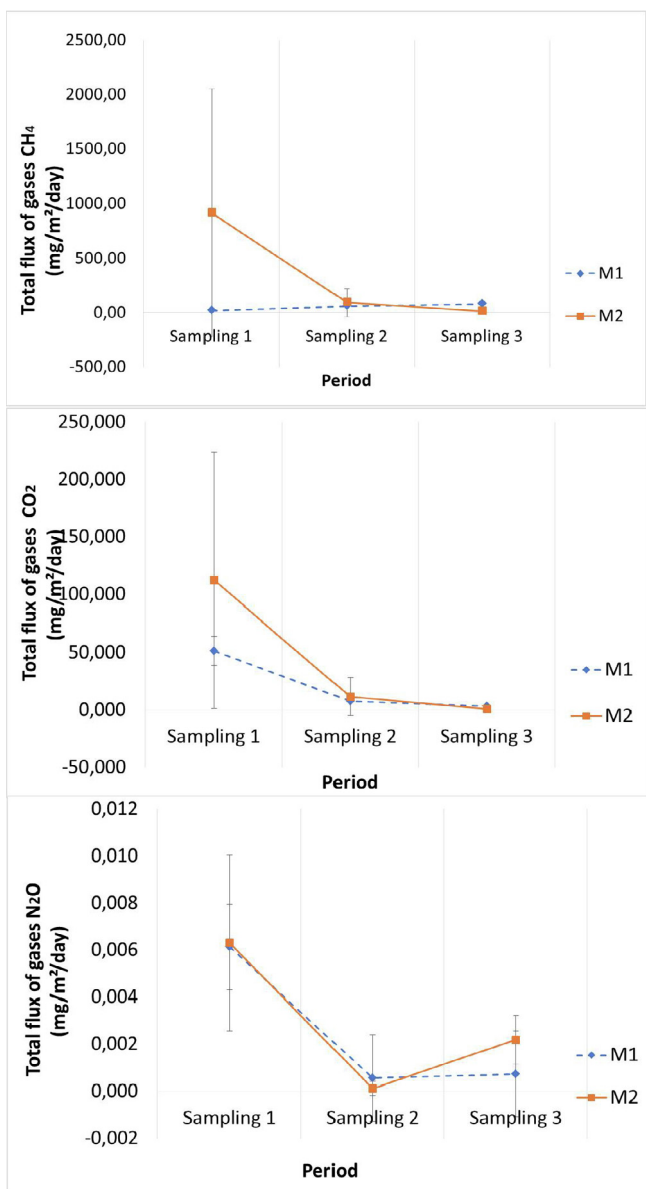


Fig. 7. Variation of gas flow and standard deviations (mg/m²/day) for the diffusive emissions between the collection periods for the two treatments. a) CH₄ – Methane, b) CO₂ – Carbon dioxide, c) N₂O – Nitrous oxide.

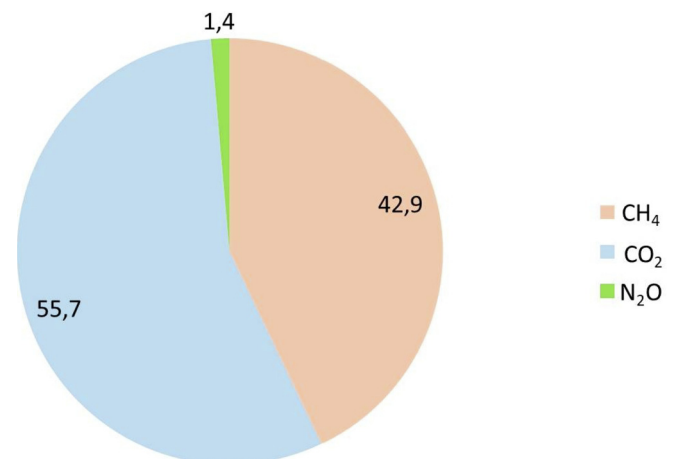


Fig. 8. Quantitative distribution of the gases produced during the study period. Sub-title: Total flux: diffusive flux + ebullient flux.

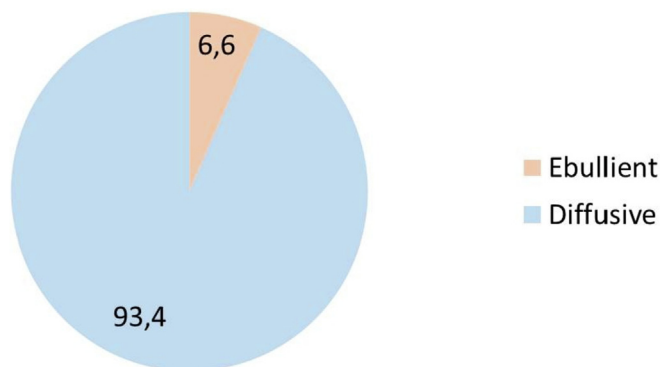


Fig. 9. Percentage contribution of the gases obtained by the two fuzzy and diffusive methods for the two treatments.

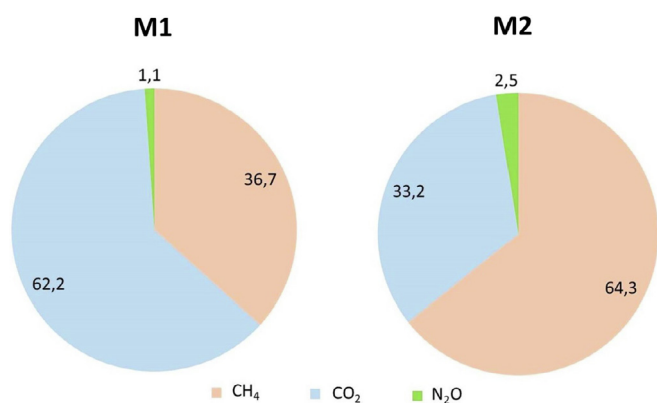


Fig. 10. Comparison of the percentage contribution of the gases obtained for the different operations performed.

capacity of these gases, such as stocking density, crop species, type of management, and water and soil characteristics in the production system.

This fact may be associated with the total water recirculation system used in the cultivation systems, which allows production in eutrophic or hypereutrophic mediums. The highest fluxes observed at the beginning of the experiment may be related to the development of an autotrophic community as the result of the initial fertilization used in the nurseries. However, a decreased variation in greenhouse gas fluxes was observed in the last sampling before the harvest, indicating the tendency to system neutrality. Considering these results and the region and spatial scale in which the experiment was developed, it is suggested that the system tends to achieve an equilibrium over time in terms of chemical, biochemical, and biological processes and can reduce gas fluxes.

The CH₄ average emission of 653.89 mg/m²/day presented in this study in M2 contrasts with the values presented in M1 (−314.9 mg/m²/day). Methane emissions in aquaculture are normally reported in positive fluxes, as observed in this study in M2 and in studies conducted by Frei and Becker (2005), who verified CH₄ concentrations of approximately 326.4 mg/m²/day in the combined cultivation *Cyprinus carpio* and rice, and 290.4 mg/m²/day in polycultures of *Cyprinus carpio* and *Oreochromis niloticus* integrated into the rice crops. Franco and Forsberg (2013) also observed similar values to those obtained by Frei and Becker (2005)

when evaluating the cultivation of *O. niloticus* in low-density tanks in the Balbina/AM hydroelectric power plant reservoir (194.81 mg/m²/day). Conversely, Preto (2012) obtained much lower results recording the mean emission of 32.55 mg/m²/day of this gas when studying the cultivation of *Macrobrachium amazonicum* in fattening nurseries with the density of 45 shrimp/m².

According to Frei and Becker (2005), there are other mechanisms that influence methane emissions, especially regarding the response of the cultivation environment to the management strategy used. The difference between the emission patterns observed between the treatments in this study can be verified because CH₄ emissions are the result of the balance between gas production by methanogenesis and oxidation from methanotrophic processes (Baggs et al., 2006; Ball et al., 1999). This effect has been mainly related to the fact that soil undergoes disturbances through the lack of fertilization and increased accumulation of organic debris (Baggs and Blum, 2004; Mojeremane et al., 2011; Suwanwaree and Robertson, 2005), which at increased concentrations may cause an increase in CH₄; while those that are less disturbed by an excess of nutrients act as a natural CH₄ drain (Chan and Parkin, 2001).

The high concentrations of dissolved oxygen in both treatments probably helped toward the low level of CH₄ emissions, as this gas may have been concentrated in anaerobic layers below the sediment (Rasenberg et al., 2013). Likewise, the high salinity can also be one of the main reasons for the low emission of CH₄. This is similar to the results from Yang and Xu (2007), who showed that in marine regions CH₄ flow becomes smaller with increasing salinity moving to the seabed. Therefore, the negative fluxes of CH₄ in M1 can be the result of the application of organic fertilizer (molasses) as the carbon source. According to Gregorich et al. (2005), any operation involving the entry of N and C into the environment can have a significant effect on the production and consumption of CH₄. Thus, the differences between the values and patterns of methane emission observed in the two studied treatments are explained by the different operating characteristics of the two cultivating environments.

The results of the CO₂ production were similar to those observed for CH₄; the results presented a mean negative flux in M1 and mean positive flux in M2, which is indicative of CO₂ absorption in M1 and CO₂ emission in M2. The main regulators of CO₂ fluxes in the aquatic environment are algae activity and organic matter mineralization (Ding et al., 2013; Tank et al., 2009). The relationship between greenhouse gas fluxes and chlorophyll-*a* corroborates this information (Yang et al., 2015) and considering that the treatment with the highest CO₂ absorption presents about twice the amount of chlorophyll-*a* detected in the other treatment. Taking into account the direct relationship between the increases in the amount of phytoplankton with CO₂ absorption, the higher amount of phytoplankton observed in M1 probably favored greater absorption of CO₂ in this treatment. Thus, this negative flow is probably due to the uptake of CO₂ by photosynthesis of phytoplankton present in the water, which exceeded the emissions of CO₂. This result indicates that the assimilation of CO₂ by phytoplankton, soil, and animals dominated the fluxes of CO₂ in M1 - a different outcome from what occurred in M2, where the concentration of CO₂ presented an increase in relation to M1.

The N₂O emission rates presented similar values between the two treatments; both presented positive flux values, indicating the emission of this gas in both treatments. These rates were lower than those observed in the other analyzed gases in both treatments. Although this is a small part of the greenhouse gas emissions

observed, it is still significant because N₂O has a high potential for global warming effects even in small amounts. The comparative impact of one N₂O unit on global warming is 280 times higher than that of CO₂. In addition, its dissipation is quite slow and can take up to 150 years (Cerri et al., 2007; Schott et al., 2016). According to Hu et al. (2012), although there are some studies on the production of N₂O in natural aquatic ecosystems such as rivers, estuaries, and the ocean, the results show that the production of N₂O in aquaculture in captivity is very high due to nitrification and denitrification processes. These results are similar to those found in wastewater treatment systems, as reported by Bicudo et al. (2015) (4.4 mg/m²/day); these results are closer to those found in our study. Thus, the amount of N₂O produced in aquaculture activities varies both as a function of the cultivated species and the cultivating environment.

Although the use of fertilizers has a direct effect on N₂O emissions, as demonstrated in many studies (Akiyama et al., 2004; Jones et al., 2007; Rochette et al., 2008), in this study, the effect was observed in a lower intensity in M1 than in M2. This result can be explained based on two aspects. According to Millar and Baggs (2004), the introduction of fertilizers such as calcium nitrate as a substrate available for the production of N, in environments where there is greater decomposition of organic matter, increases the production of N₂O, which has been demonstrated in the results observed in M2. The other aspect would be the control N₂O fluxes through the addition of C in the environment through the use of an organic fertilizer (molasses), which is known for stimulating denitrification (Jones et al., 2007), and which would justify the low N₂O flux detected in M1.

Some studies have demonstrated variations of gas emissions in different animal production activities. In general, estimates of

greenhouse gas emissions in animal production show a wide range of variation due to the complexity of production systems and their unpredictability (due to their unstable animal component). Table 4 presents comparative data on the emissions of the main greenhouse gases (CO₂, CH₄, and N₂O) reported in studies conducted with animals in confinement (livestock, swine, poultry, and aquaculture) and in studies in water reservoirs set up for electricity production.

Comparing all activities presented in Table 4, it can be observed that the scenario of lowest gas emissions was verified in poultry farming. As expected, livestock production is the highest CH₄ emitter (Berchielli et al., 2013; Lage et al., 2012), though not presenting representative emissions of other gases. Among aquaculture activities, shrimp farming (present study) presents the highest N₂O emission, which is only lower than emissions observed in sugar cane crops. This result is relevant because N₂O represents the gas with the greatest potential for global warming, even when emitted in small amounts.

Table C.1 shows the main evaluated greenhouse gases (CO₂, CH₄, and N₂O) assembled by their global warming potential over 100 years. These results are expressed in carbon equivalent (CO₂-eq), which is a measure used to compare emissions of various greenhouse gases based on their global warming potential (GWP). CO₂-eq is the result of the multiplication of tons of greenhouse gases (GHG) emitted by their global warming potential (IPAM, 2013). These results are presented in descending order of total emissions of greenhouse gases. For comparison purposes, the values of gases referring to different agricultural production systems such as livestock, swine, poultry, sheep, and shrimp farming were considered. The total level of greenhouse gas emissions in animal production, in

Table 4
Comparative values on the emissions greenhouse gases (mg.m².day⁻¹) in different activities.

ACTIVITIES	Gases (mg.m ² .day ⁻¹)			AUTHORS
	CH ₄	CO ₂	NO ₂	
ANIMAL PRODUCTION				
Livestock	119 400			Berchielli et al. (2013)
Livestock	142 600			Lage et al. (2012)
Swine	816	10 944	5.76	Amorim et al. (2013)
Poultry	4.22	40.69	2.67	Santana (2016)
AQUACULTURE – FISH FARMING				
<i>O. niloticus</i> ¹	194.81	6354		Franco & Forsberg (2013)
<i>O. niloticus</i> ¹			0.28	Ferreira et al. (2014)
<i>C. idella</i> ² + <i>H. molitrix</i> ³		97.8		Chen et al. (2015)
<i>C. carpio</i> ⁴ + rice	326.4			Frei and Becker (2005)
<i>C. carpio</i> ⁴ + Rice + <i>O. niloticus</i> ¹	290.4			Frei and Becker (2005)
<i>C. fuscus</i> ⁵			0.65	Paudel et al. (2015)
AQUACULTURE – SHRIMP FARMING				
<i>M. amazonicum</i> ⁶	32.55	1346.76		Preto (2012)
<i>L. vannamei</i> (M1) ⁷	-314.9	-3773.5	2.5	Present Work
<i>L. vannamei</i> (M2) ⁷	653.9	497.5	25.5	Present Work
AGRICULTURE				
Rice cultivation	245.8			Costa (2005)
Rice cultivation	229.1			Lima et al. (2002)
Rice cultivation	256.8			Frei e Becker (2005)
Sugar cane (with fertilizers)		11 330	53.54	Degaspari et al. (2013)
Sugar cane (natural soil)		9660	5.2	Degaspari et al. (2013)
Eucalyptus (with fertilizers)	57.53			Souza et al. (2013)
Eucalyptus (natural soil)	-43.8			Souza et al. (2013)
AQUATIC ENVIRONMENTS				
Hydropower (Amazon region)	196	84 574.5		Faria et al. (2015)
Hydropower (Itaipu)	10.7	170		Faria et al. (2015)

¹*Oreochromis niloticus*, ²*Ctenopharyngodon idella*, ³*Hypophthalmichthys molitrix*, ⁴*Cyprinus carpio*, ⁵*Clarias fuscus*, ⁶*Macrobrachium amazonicum*, ⁷*Litopenaeus vannamei*, CH₄ – Methane, CO₂ – Carbon dioxide, N₂O – Nitrous oxide.

kg CO₂-eq/kg of meat, was higher in the production of livestock, and sheep and shrimp farming in M2 (with 18.8, 14, and 11.6 kg of CO₂-eq per kg of meat, respectively), and lower in shrimp farming using M1, and in swine and poultry farming (with 6.2, 3.6, and 2.1 kg of CO₂-eq per kg of meat, respectively). If only N₂O is considered, the gas with the highest greenhouse potential, the highest emissions in kg CO₂-eq/kg of meat were observed in sheep, shrimp, and livestock farming, respectively.

Table D.1 shows an estimate of global N₂O emissions up to the year of 2030 in different groups of species cultivated in aquaculture. These data show the dominance of N₂O production in the cultivation of shrimp and other crustaceans over other species, reaching about half of the estimated amount of global emission of N₂O by 2030 produced by aquaculture activities, and exemplifying the high potential of shrimp farming to contribute to N₂O emission.

5. Conclusion

Considering the emissions of greenhouse gases in different activities of the agricultural sector, it can be observed that livestock production continues to be the main source of gas emissions in quantitative terms, especially represented by CH₄. However, emissions from shrimp farming are potentially critical, mainly due to N₂O emissions, because this gas has a high contributing potential for global warming. Conversely, shrimp farming, particularly those operations carried out using organic fertilizers such as molasses, showed potential for the absorption of gases such as CH₄ and CO₂. In this study, the highest gas fluxes were detected at the beginning of the experiment as the result of the initial fertilization. However, these fluxes tended to neutrality in the system due to the equilibrium of chemical, biochemical, and biological processes over time. It can be concluded from the present study that the M2 treatment used in the cultivation of *L. vannamei* leads to the emission of more greenhouse gases than M1. The difference in the CO₂ flux was probably related to the effects of photosynthesis, biological respiration and the mineralization of organic matter, whereas the N₂O fluxes were controlled by the interactions between nitrogen substrate availability. Water salinity, trophic status and dissolved oxygen concentration probably affected CH₄ emission. The results of this study demonstrate that the management of a system producing *L. vannamei* can influence the fluxes of greenhouse gases. The use of molasses, which tends to favor denitrification and increase the natural productivity through fertilization, may have contributed to the lower emission of gases observed compared to the emissions in the treatment that did not use molasses; this conclusion was supported by the high values of chlorophyll-*a* measured in that treatment. Considering the several factors that may influence the emission of greenhouse gases in shrimp farming, it is suggested as future research, to evaluate each factor separately and to verify the interaction between them in order to identify the main indicators of this activity that favor the emission these gases and if any interaction with other factors can provoke positive or negative reactions.

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List of abbreviation, parameters and variables

CH ₄	methane
CO ₂	carbon dioxide
N ₂ O	nitrous oxide
ORP	oxidation reduction potential
DO	Dissolved oxygen
TDS	total dissolved solids
TC	Total Carbon
TIC	Total Inorganic Carbon
TOC	Total Organic Carbon
OM	organic matter
M1	management systems 1
M2	management systems 2
AFC	apparent feed conversion factor
CP	Percentage crude protein rations
P	Percentage of phosphorus rations
E.E	Percentage of ethereal extract
GHG	greenhouse gases
GWP	Global warming potential
CO ₂ -eq	carbon equivalent

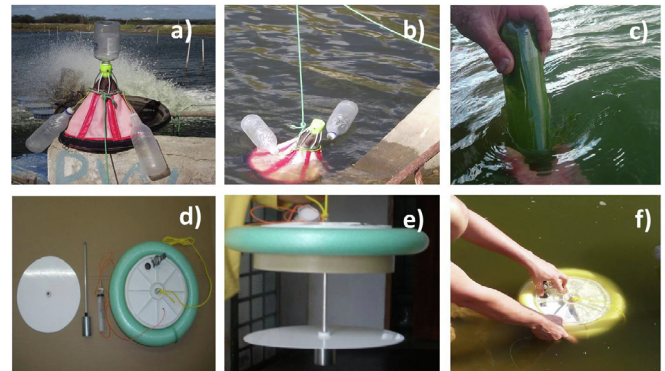


Fig. A.1. Gas collection equipment. (a) Invert funnel with a diameter of 0.0707 m², used to capture ebullient bubbles - photographed out of water. (b) Installation of funnels. (c) Collecting ebullient gases samples. (d) Diffusion chambers - device to measure gas emanation rates for air - photographed out of water. (e) Disassembled diffusion chamber. (f) Collecting diffusive gases samples.

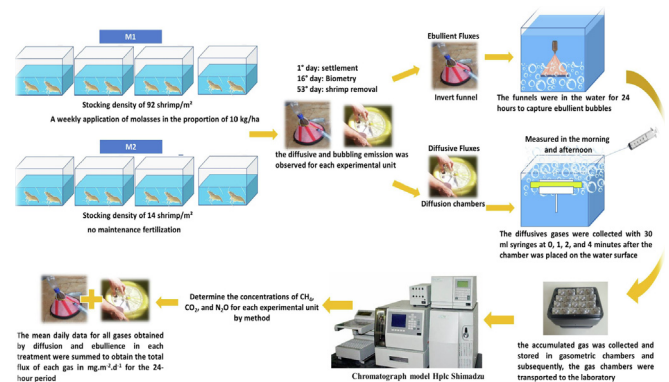


Fig. B.1. Workflow for cultivation, management and analysis of shrimp culture to evaluate the emission of greenhouse gases produced by this activity.

Table C.1Emissions Greenhouse gases referring to different agricultural production systems in kg CO_{2-eq}/kg of meat evaluated and assembled by their global warming potential over 100 years.

GAS	Nemry et al. (2001)				Present study				Nemry et al. (2001)			
	Sheep		Livestock		Shrimp farming		Shrimp farming		Swine		Poultry	
					(M2)		(M1)					
	[1]	[2]	[1]	[2]	[1]	[2]	[1]	[2]	[1]	[2]	[1]	[2]
CH ₄	7.6	40.5	6.3	42.4	3.6	30.8	1.7	27.7	1.7	46.2	0.7	31.1
CO ₂	1.9	9.9	3.4	23.2	0.5	4.3	3.8	60.6	0.9	24.7	0.8	37.4
N ₂ O	9.3	49.6	5.1	34.5	7.5	64.9	0.7	11.7	1.1	29.1	0.7	31.5
Total	18.8	100	14.8	100	11.6	100	6.2	100	3.6	100	2.1	100

CH₄ – Methane, CO₂ – Carbon dioxide, N₂O – Nitrous oxide, [1] emissions kg CO_{2-eq}/kg of meat, [2] percentage distribution between the three gases considered. Adapted from Nemry et al. (2001), from report to the “Global Change and Sustainable Development” Program.

Table D.1Estimated global emission of N₂O in 2030

Species group	Estimated N ₂ O emission in 2030 (metric ton)
Carp, barbels, cyprinids	86997
cods, hakes	647
ztunas, bonitos	29
salmons, trouts	36547
Tilapias	17271
Sturgeons	7629
Shrimp	63235
Crabs	853
other crustaceans	61235
Oysters	9922
Mussels	4501
Scallops	7791
TOTAL	296657

Table based on data published in the Fisheries of the United States report on world aquaculture (2009) (Adapted of the Hu et al., 2012).

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