



## Ecotoxicological and physicochemical evaluation of an effluent of a shrimp farm located in Northeastern Brazil

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**Abstract:** Aquaculture produces effluents which negatively impact the environment. Quality control of such effluents is generally undertaken via the analysis of physical and chemical parameters; however, such an isolated approach does not provide data on the effects on the organisms exposed to the effluents. Thus, ecotoxicological bioassays are used to assist in traditional physical-chemical determinations to complement chemical data. This study analyzed physicochemical variables, dissolved nutrients, chemical oxygen demand (COD), and toxicity of a shrimp farm effluent in the State of Ceará, Brazil. Values of physical-chemical parameters and dissolved inorganic nutrients analyzed in the effluent remained similar to those found in other studies, except for orthophosphate, which presented higher levels. Only ammonia and dissolved oxygen presented variations over the sampling period, while the other parameters analyzed did not present relevant variations. The LC<sub>50</sub> ranged from 7.37 to 18.91%, indicating potential toxicity of the effluent, particularly in April (LC<sub>50</sub> 7.37%). This high toxicity could be due to the impact of runoff, which inserts pollutants, such as heavy metals, into the sedimentation basin.

**Keywords:** Shrimp, Water, Quality, Mysid, Ecotoxicology.

**Resumo. Avaliação ecotoxicológica e físico química do efluente de uma fazenda de camarão no Nordeste do Brasil.** A aquicultura produz efluentes que afetam negativamente o meio ambiente. O controle de qualidade de tais efluentes é geralmente realizado através da análise de parâmetros físicos e químicos. No entanto, tal abordagem isolada não fornece dados sobre os efeitos nos organismos expostos aos efluentes. Assim, os bioensaios ecotoxicológicos são utilizados para auxiliar as determinações físico-químicas tradicionais, a fim de complementar os dados químicos. Este estudo analisou as variáveis físico-químicas, os nutrientes dissolvidos, a demanda química de oxigênio (DQO) e a toxicidade de um efluente de cultivo de camarão no Estado do Ceará. Os valores dos parâmetros físico-químicos e dos nutrientes inorgânicos dissolvidos analisados no efluente permaneceram semelhantes aos encontrados em outros estudos, com exceção do ortofosfato, que apresentou níveis mais elevados. Apenas amônia e oxigênio dissolvido apresentaram variação no período de amostragem, enquanto os demais parâmetros analisados permaneceram semelhantes. A CL<sub>50</sub> variou de 7,37 a 18,91%, indicando potencial toxicidade do efluente, em particular no mês de abril (CL<sub>50</sub> 7,37%). Esta alta toxicidade pode ser devida ao impacto do escoamento, que insere poluente, como metais pesados, na bacia de sedimentação.

## Palavras-Chaves: Camarão, Água, Qualidade, Misidáceo, Ecotoxicologia

### Introduction

The development of aquaculture in the last decade was motivated by high population growth rates and an increased global demand for animal protein. Studies show that such growth is expected to continue (Gutierrez-Wing & Malone 2006; Fao 2011). This development has brought benefits to countries implementing aquaculture (Fao 2011), including the creation of new jobs, the maintenance of wild stock fisheries, and high profitability for aquaculture farmers.

Despite these benefits, the development of aquaculture is accountable for a variety of environmental impacts. Aquaculture farm effluents are rich in dissolved inorganic nutrients, organic matter, and suspended solids (Hopkins *et al.* 1995; Páez-Osuna 2001; Boyd 2003; Hussenot 2003). Enrichment of the effluent is the result of an incomplete conversion of food supplied to cultured organisms. Only 30% of the nitrogen and phosphorus contained in the food composition is incorporated into the shrimp, the rest being released into the water. Besides that, feces and non-metabolized food are also present in an effluent and have potentially harmful effects on the environment (Shimoda *et al.* 2007).

Effluents released from aquaculture farming often present higher levels of ammonia, nitrite, nitrate, and phosphate compared with the influent (Mcintosh & Fitzsimmons 2003; Samocha *et al.* 2004), suggesting that aquaculture significantly contributes to rising levels of nutrients in the environment. In addition, other studies have demonstrated the presence of heavy metals, pesticides and emerging pollutants in aquaculture effluents (Gräslund *et al.* 2003). Excess nutrients and organic matter in aquaculture wastewater result in decreased water quality in coastal environments, such as estuaries (Hopkins *et al.* 1995; Ferreira *et al.* 2011). To avoid such impacts, aquaculture farmers are required to use ponds to discharge wastewater before releasing it into the receiving water body. Nevertheless, although sedimentation tanks can somehow mitigate the amounts of suspended solids, amounts of dissolved inorganic nutrients and organic matter can remain unaltered (Hussenot 2003). Therefore, the adoption of improved management practices is necessary to prevent

water pollution and guarantee resource maintenance (Boyd 2003).

The measurement of physical-chemical parameters is frequently used to evaluate effluent quality. However, these parameters only indicate a potential degradation caused by the effluent, but not the respective effects on aquatic organisms and ecosystems (Stephens & Farris 2004). In this context, toxicity tests are the methods of choice (Rand 1995).

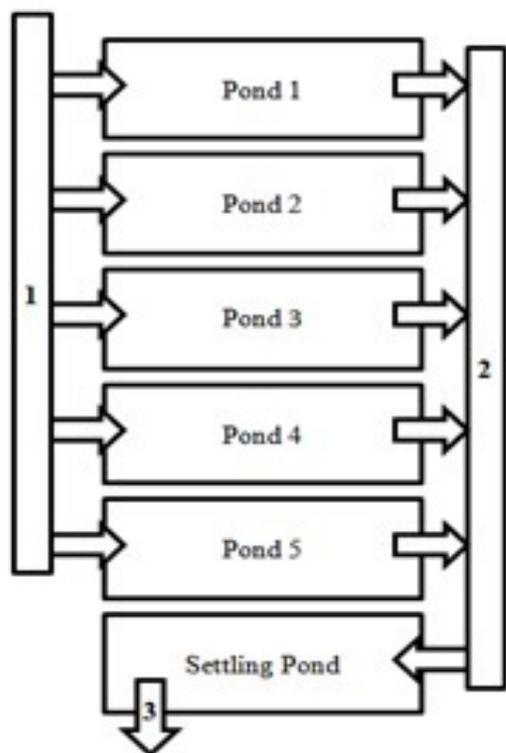
Brazil is among the world's 10 largest shrimp producers, with a production of 64,669 tons in 2013. Most of the shrimp production is in Northeastern Brazil, with the state of Ceará being one of the largest national producers (66.7% of annual production). The state of Ceará has 245 legalized shrimp farms covering an area of approximately 6,069 ha, with the majority being located in mangrove swamps (Ibama 2005).

Given that, the present study aimed to assess the quality of effluent released from shrimp farm ponds by evaluating the temporal variation of physical-chemical parameters and toxicity to non-target organism.

### Materials and Methods

*Shrimp farm operations and sampling:* Water samples were collected from a shrimp farm located in Itapipoca (west coast of Ceará, Brazil), which is 130 km away from Fortaleza, the capital of the state of Ceará (03°43'02"S/38°32'35"W). The west coast region has circa 94 shrimp farms, most of which are small and medium businesses, with approximately 2458.23 ha of area for cultivation. Shrimp farms are distributed in the coastal region near the estuarine region to facilitate the collection of saline water. The intensive shrimp farm is composed of five rearing ponds aerated and stocked with *Litopenaeus vannamei* (Crustacea: Decapoda) at a density of 35 animals m<sup>-2</sup> and one sedimentation pond (Fig. 1). During cultivation, commercial feed containing 30-40% protein is added to the ponds daily. Excess water is continuously discharged into the environment during the rearing period.

*Chemical analyses:* Water parameters – pH, salinity, dissolved oxygen (DO), total suspended solid (TSS), unionized ammonia (NH<sub>3</sub>), nitrite (NO<sub>2</sub><sup>-</sup>), nitrate (NO<sub>3</sub><sup>-</sup>), orthophosphate (PO<sub>4</sub><sup>3-</sup>), chlorophyll-a (Chl-a), and chemical oxygen demand (COD) – were analyzed in the laboratory. Salinity, pH, and DO were measured with the aid of specific instruments.



**Figure 1.** Schematic layout of the shrimp farm analyzed in this study. (1) Supply channel, (2) Drainage channel and (3) Output of the settling pond.

Table I summarizes the methods used in this study.

**Acute toxicity test:** The microcrustacean *Mysidopsis juniae*, which was cultured in laboratory, was used in the toxicity test. The microcrustaceans were maintained under controlled conditions (salinity  $35 \pm 2$ ; photoperiod of 12:12, light:dark; temperature of  $25 \pm 2^\circ\text{C}$ ; constant aeration) in aquariums (10 L) with filtered natural seawater (filter  $0.8 \mu\text{m}$ ). Mysids were fed microcrustacean *Artemia* sp. (72 h) enriched with fish oil daily *ad libitum*.

The test concentrations (6.25, 12.5, 25, 50, and 100%) were obtained by effluent dilution with filtered natural seawater – the same used in control. Ten juvenile mysids (six to eight days

old) were exposed to effluent samples (300 ml) in quadruplicates for a period of 96 h. The organisms were counted daily and the dead ones (immobilized for at least 10 sec) were removed. Subsequently, the remaining mysids were fed *Artemia* sp. (48 h) without fish oil. Physical-chemical parameters (pH, salinity, and OD) were measured at the beginning and at the end of the experiment. All samples were submitted to a toxicity test.

**Statistical analyses:** Data were grouped and submitted to normality test (Shapiro-Wilk test). Subsequently, Spearman’s correlation test ( $p < 0.05$ ) was used to assess correlations between chemical parameters. One-way ANOVA with Tukey post hoc was used to identify significant differences in all variables between the different sampling months. Data which did not pass the normality test were submitted to Kruskal-Wallis’s test with Dunn post hoc to check for significant differences between different months. The lethal concentration 50 ( $LC_{50}$ ) was estimated using the Trimmed Spearman-Kärber method (Hamilton et al., 1970). All analyses were performed using GRAPHPAD PRISM 5.

**Results**

**Chemical analyses:** Table II shows the physical-chemical characterization of effluents in each sampling period. In all, the variables presented little variation throughout the experimental period, except for salinity, TSS,  $\text{PO}_4^{3-}$ , COD, and Chl-*a*, which presented greater variation. The data for the month of April were excluded from the statistical analyzes, since only one single collection was possible in this period.

Statistically significant differences between months were found only in DO and  $\text{NH}_3$  (The data for April were excluded from the statistical analyzes because sampling could only be carried out once in such period). The parameter DO presented the lowest value in May ( $5.15 \text{ mg L}^{-1}$ ) when compared with the other months. Mean concentration of  $\text{NH}_3$  was significantly higher in June ( $2.59 \text{ mg L}^{-1}$ ) compared with March and May.

**Table I.** Analyses of water quality parameters

Variable	Method	Detection limit	Reference
$\text{NH}_3$	Distillation nitrogen – (4500-NH <sub>3</sub> B)	-	Eaton et al. (2005)
$\text{NO}_2^-$	Diazotization	$0.005 \text{ mg L}^{-1}$	Baugarten et al. (1996)
$\text{NO}_3^-$	Cadmium reduction column – (4500-NO <sup>3-</sup> C)	$0.01 \text{ mg L}^{-1}$	Eaton et al. (2005)
$\text{PO}_4^{3-}$	Ascorbic acid	$0.05 \text{ mg L}^{-1}$	Baugarten et al. (1996)
Chl- <i>a</i>	Extraction in 90% acetone (10200-H)	-	Eaton et al. (2005)
COD	Potassium chromate method (5220-D)	$25 \text{ mg L}^{-1}$	Eaton et al. (2005)
TSS	Gravimetric	-	Silva & Oliveira (2001)

**Table II.** Means  $\pm$  SD for physical-chemical parameters and effluent toxicity. Equivalent superscript letters in the same row indicate absence of significant difference between months. Different superscript letters in the same row indicate significant difference between months.

Parameters	March	April <sup>1</sup>	May	June
pH	7.91 $\pm$ 0.3 <sup>a</sup>	7.76	7.73 $\pm$ 0.2 <sup>a</sup>	7.98 $\pm$ 0.0 <sup>a</sup>
Salinity	37.0 $\pm$ 5.6 <sup>a</sup>	26	21 $\pm$ 8.4 <sup>a</sup>	24 $\pm$ 8.4 <sup>a</sup>
DO (mg L <sup>-1</sup> )	9.14 $\pm$ 0.6 <sup>a</sup>	7.09	5.15 $\pm$ 0.1 <sup>b</sup>	8.50 $\pm$ 0.6 <sup>a</sup>
TSS (mg L <sup>-1</sup> )	222.5 $\pm$ 75.4 <sup>a</sup>	165.8	117.1 $\pm$ 44.1 <sup>a</sup>	149.5 $\pm$ 46.5 <sup>a</sup>
NH <sub>3</sub> (mg L <sup>-1</sup> )	0.35 $\pm$ 0.0 <sup>a</sup>	2.8	1.33 $\pm$ 0.2 <sup>ab</sup>	2.59 $\pm$ 0.6 <sup>b</sup>
NO <sub>2</sub> <sup>-</sup> (mg L <sup>-1</sup> )	0.01 $\pm$ 0.0 <sup>a</sup>	0.02	0.01 $\pm$ 0.0 <sup>a</sup>	0.01 $\pm$ 0.0 <sup>a</sup>
NO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	0.0002 $\pm$ 0.0 <sup>a</sup>	0.003	0.003 $\pm$ 0.0 <sup>a</sup>	0.0015 $\pm$ 0.0 <sup>a</sup>
PO <sub>4</sub> <sup>3-</sup> (mg L <sup>-1</sup> )	86.52 $\pm$ 38.9 <sup>a</sup>	194.1	133.2 $\pm$ 34.6 <sup>a</sup>	168.0 $\pm$ 12.2 <sup>a</sup>
Chl- <i>a</i> (µg L <sup>-1</sup> )	4.99 $\pm$ 3.3 <sup>a</sup>	1.069	1.06 $\pm$ 0.7 <sup>a</sup>	0.80 $\pm$ 0.3 <sup>a</sup>
COD (mgO <sub>2</sub> L <sup>-1</sup> )	420.8 $\pm$ 370.1 <sup>a</sup>	17.98	36.68 $\pm$ 24.1 <sup>a</sup>	182.4 $\pm$ 49.3 <sup>a</sup>

*Acute toxicity:* The results of the toxicity test showed that the effluent samples were highly toxic in all the months. The mortality of the organisms in the concentrations tested was significantly different from that of the control (Fig. 2). In April and May, effluents presented higher toxicity. In June, toxicity was lower (Fig. 3). In addition, precipitation was relatively high in March (110 mm), May (105 mm), and April (228 mm). In June, rainfall decreased by approximately 95% compared with April (10.2 mm).

*Correlations between water quality parameters:* The Spearman's correlation test (Table III) showed that COD was negatively correlated to nitrite. The parameters DO and COD were positively correlated; similarly, there was a positive correlation between pH and acute toxicity. Nitrate was correlated to nitrite, COD, DO, chlorophyll-*a*, and salinity. The highest correlation value found was between Salinity and TSS.

## Discussion

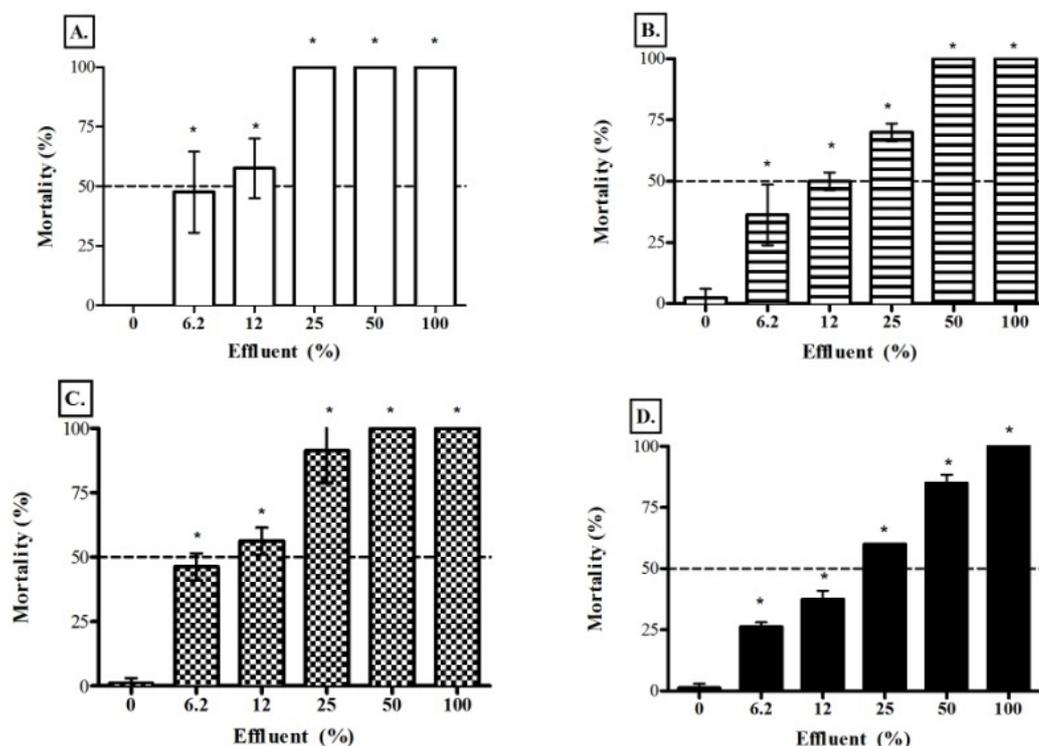
*Salinity, dissolved oxygen, and pH:* The salinity levels measured in the present study are consistent with those reported in other studies on aquaculture effluents, which ranged from 15 to 25 (Alves & Mello 2007; Herbeck *et al.* 2013). In May, the dissolved oxygen levels were similar to values found by other authors, usually around 4 mg L<sup>-1</sup> (Shireman & Cichra 1994; Mcintosh & Fitzsimmons 2003; Stephens & Farris 2004; Ferreira *et al.* 2011).

Levels of oxygen in the water column above 4 mg L<sup>-1</sup> are essential for the maintenance of aquatic ecosystems (Alam *et al.* 2007). Metabolic and behavioral changes in fish populations and a decrease in the benthic invertebrate population are likely to occur at values below 4 mg L<sup>-1</sup> (Karna 2003).

Water pH showed no variation (7.73–7.98) over the months; additionally, its values were similar to those found in effluents from other shrimp farms (Samocha *et al.* 2004; Alves & Mello 2007; Herbeck *et al.* 2013).

*Nutrients:* The ammonia concentrations determined in the present study (0.35–2.8 mg L<sup>-1</sup>) were similar to those reported by Samoncha *et al.* (2004) in a shrimp farm and by Shireman & Cichra (1994) and Stephens & Farris (2004) in fish farms. However, lower values – 0.04–0.224 mg L<sup>-1</sup> – have also been determined in shrimp farm effluents (Mcintosh & Fitzsimmons 2003; Samocha *et al.* 2004; Ferreira *et al.* 2011). The ammonia levels found in the effluent from the shrimp farm analyzed in the present study are higher than some values reported in other studies (Shireman & Cichra 1994; Samocha *et al.* 2004), possibly due to the density adopted by the farm – 35 shrimp/m<sup>2</sup> in the ponds. An animal density of 15 shrimps/m<sup>2</sup> (Samocha *et al.* 2004; Ferreira *et al.* 2011) and a high feed conversion rate (Mcintosh & Fitzsimmons, 2003) resulted in lower ammonia concentrations compared with those in the present study. High-density rearing, a common practice in shrimp farming, requires the use of large amounts of feed, often resulting in the release of high amounts of excreta (Thakur & Lin 2003; Shimoda *et al.* 2007).

The nitrite values in the present study were similar to those reported in the literature on shrimp farm effluents, ranging from 0.003 to 0.054 mg L<sup>-1</sup> (Shireman & Cichra 1994; Stephens & Farris 2004; Herbeck *et al.* 2013). Nitrite is usually present in water bodies at low concentrations, mainly due to the slow growth of bacteria belonging to the genus *Nitrosomonas*, which are responsible for the nitrification of ammonia (Sperling 2007). Furthermore, nitrite is an



**Figure 2.** Effect of effluent on mysid survival after 96 h of exposure. Upper case letters indicate collection months: A – March; B – April; C – May; D – June. The control group is represented by the number 0.

intermediate compound of nitrification and denitrification and is therefore rapidly converted into nitrate or ammonia, respectively (Philips *et al.* 2002).

The nitrate concentration was also low compared with other studies that have presented nitrate levels between 0.03 and 9.80 mg L<sup>-1</sup> (Shireman & Cichra 1994; Mcintosh & Fitzsimmons 2003; Samocha *et al.* 2004; Stephens & Farris 2004; Ferreira *et al.* 2011; Herbeck *et al.* 2013). In the rainy season, Stephens & Farris (2004) measured the lowest effluent nitrate levels (0.03mg L<sup>-1</sup>), a pattern that was also observed in the present study.

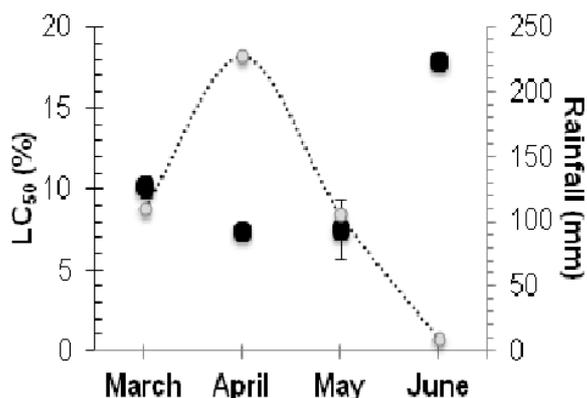
The low levels of nitrate and nitrite compared with ammonia can be related to the small number of bacteria responsible for nitrification and to the high ammonia input rates resulting from uneaten or unmetabolized food, which release ammonia after being mineralized in water column.

In the present study, orthophosphate concentrations were higher than those found in other studies on aquaculture effluents (Mcintosh & Fitzsimmons 2003; Samocha *et al.* 2004; Stephens and Farris 2004; Ferreira *et al.* 2011). Herbeck *et*

*al.* (2013) found high orthophosphate levels (130.2 mg L<sup>-1</sup>) in the drainage channels of aquaculture ponds; these values are similar to the concentrations found in the present study. Such high levels could be attributed either to excess feed or organic matter mineralization. The level of orthophosphate found in the effluent analyzed in the present study may be associated with excess food.

Effluent storage in sedimentation basins prior to release into the environment may be responsible for the high levels of orthophosphate in the water discharged. Microalgae may play a role in the senescence process due to the low amounts of nitrate (essential nutrient with easy uptake) in the water (Boyd & Tucker 1998; Fernandes *et al.* 2005; Chester 2012), resulting in the release of orthophosphate due to phytoplankton decomposition (Hargreaves, 1998).

**Total suspended solids (TSS):** The levels of total suspended solids (TSS) were similar to those found in the literature, ranging from 85.80 to 275.80 mg L<sup>-1</sup> (Mcintosh & Fitzsimmons 2003; Samocha *et al.* 2004; Stephens & Farris 2004). High and low levels are found in the initial and



**Figure 3.** Variation in LC<sub>50</sub> values according to the rainfall at collection points. Black squares indicate LC<sub>50</sub> values; grey circles indicate rainfall.

final harvest period, respectively (Stephens & Farris 2004).

Residues of organic and inorganic matter, resuspended sediment, and metabolic waste of animals are responsible for high TSS values. In water bodies, TSS may reduce light penetration, change water temperature and alter deposition of nutrients and pesticides via adsorption (Bilotta & Brazier 2008).

**Chlorophyll-a:** Chlorophyll-*a* values found in the effluent were lower than the values found in other studies (Shireman & Cichra 1994; Stephens & Farris 2004; Herbeck *et al.* 2013), which reported concentrations of up to 80,000 µg L<sup>-1</sup>.

The long hydraulic retention time of the effluent in the sedimentation basin may have contributed to the low amounts of chlorophyll-*a*. In laboratory studies, a 45.1% chlorophyll-*a* removal could be achieved using a hydraulic retention time of six hours (Ramos *et al.*, 2009).

In addition, nutrient availability for the development of phytoplankton biomass can be

influenced by low values of chlorophyll-*a*, as seen in the low nitrate concentrations of the effluent. Nitrate is an essential nutrient that is easily assimilated by microalgae; low nitrate concentrations limit phytoplankton growth (Fernandes *et al.* 2005; Glibert *et al.* 2015).

**Chemical oxygen demand:** Values of COD ranged from 17.98 to 682.52 mg O<sub>2</sub> L<sup>-1</sup>. McIntosh & Fitzsimmons (2003) observed significantly lower COD values, with a maximum of 88 mgO<sub>2</sub> L<sup>-1</sup>. In a similar study carried out along the drainage channel of a shrimp farm by Biao *et al.* (2004), the values of COD did not exceed 10 mg O<sub>2</sub> L<sup>-1</sup>.

Aquaculture produces effluents with high loads of organic matter. This organic matter load requires a certain amount of oxygen to be degraded, which results in the reduction of dissolved oxygen (Boyd 1998). In aquaculture ponds, such depletion of dissolved oxygen is not observed due to constant mechanical aeration.

The high COD (oxygen required to oxidize organic matter in the water) values found for the analyzed effluent are in disagreement with the high levels of OD. The high DO values found in the studied effluent can be attributed to the presence of aerators in shrimp ponds. In addition, the outlet arrangement of the sedimentation basin effluent may also have contributed to the high DO value. The outlet of the tank formed a slope that stirred the water and broke the surface tension of water and thus facilitated oxygenation of the water.

**Toxicity test:** The effluent had a lethal effect on the mysid *M. juniae*. Even at relatively low concentrations, the effluent had a significant deleterious effect. The NOEC (No Observed Effect Concentration) values for all samples were below the lowest concentration tested (6.25%). These results show that shrimp farm effluent is extremely toxic.

**Table III.** Correlation matrix of the parameters analyzed in the effluent from the shrimp farm. Values in bold indicate significant correlations ( $p < 0.05$ ).

	NH <sub>3</sub>	NO <sub>2</sub> <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	PO <sub>4</sub> <sup>3-</sup>	COD	pH	DO	Sal.	TSS	Tox.	Chl- <i>a</i>
NH <sub>3</sub>	1	0.107	0.400	0.500	-0.321	0.0357	-0.179	-0.607	-0.429	0.321	-0.519
NO <sub>2</sub> <sup>-</sup>		1	<b>0.782</b>	0.900	<b>-0.750</b>	-0.250	-0.607	-0.429	-0.286	-0.607	-0.408
NO <sub>3</sub> <sup>-</sup>			1	0.718	<b>-0.891</b>	-0.491	<b>-0.782</b>	<b>-0.764</b>	-0.600	-0.546	<b>-0.774</b>
PO <sub>4</sub> <sup>3-</sup>				1	-0.700	-0.100	-0.100	-0.600	-0.300	-0.500	-0.447
COD					1	0.643	<b>0.857</b>	0.571	0.464	0.714	0.704
pH						1	0.571	0.536	0.643	<b>0.821</b>	0.148
DO							1	0.500	0.536	0.571	0.704
Sal.								1	<b>0.929</b>	0.286	0.445
TSS									1	0.321	0.259
Tox.										1	0.111
Chl- <i>a</i>											1

The  $LC_{50}$  values ranged from 7.37 to 18.91%, with the highest toxicity being recorded in April and the lowest in June – highest and lowest rainfall, respectively. This high toxicity could be a result of the runoff that inserted other pollutants, adsorbed on soil particles or plants, into the sedimentation basin, thus contributing to increased effluent toxicity during rainy periods. Zhou *et al.* (2015) observed that runoff was one of the main factors that reduced the water quality by increasing nitrogen and phosphorus levels. In another study, runoff was responsible for the increase of heavy metals in the water column, particularly Pb, Cu, and Fe (Suratman *et al.* 2009).

Sediment remobilization, caused by heavy rains, may also be related to the observed toxicity, as sedimentation basins are likely to accumulate compounds such as metals (Han *et al.* 2001; Huggett *et al.* 2001) which, when available in the water column, may have sublethal and lethal effects on non-target organisms (Oliveira *et al.* 2014; Yang 2014).

There are few studies on the toxic effects of aquaculture effluents on aquatic organisms. Typically, these studies prioritize the determination of physicochemical parameters, thus excluding the ecotoxicological approach. However, studies that measure physical-chemical parameters and use ecotoxicological tests in effluent analyses are considered more robust since they show the possible impact of these discharges on the environment.

Stephens and Farris (2004) observed acute effects of aquaculture effluent on *Pimephales promelas* (fish) and *Ceriodaphnia dubia* (microcrustacean); fish survival rate was reduced by 20.50 and 2.60% when exposed to the effluent released at the beginning and at the end, respectively, of the fish removal period. The survival rate of *C. dubia* decreased 12.80% when it was exposed to the effluent generated at the beginning of the shrimp harvest; however, the final effluent had no acute toxic effects. Another study with *C. dubia* (Moreira *et al.* 2010) found an  $LC_{50}$  of 56.62%, with the influent presenting the highest toxicity. Aragão (2006) analyzed shrimp farm effluent toxicity on Ceará coast using the same model organism (*M. juniae*) and obtained  $LC_{50}$  values of 5.12 to 46.9 %, which are similar to the ones found in the present study.

Other chemicals used in aquaculture can have adverse effects on non-target organisms (Sapkota *et al.* 2008; Shamsuzzaman *et al.* 2012; Sharkar *et al.* 2014; Macken *et al.* 2015). The use

of fertilizers, pesticides, disinfectants, antibiotics and immune stimulants is common (Graslund *et al.* 2003), and metabolism products excreted by the organisms may remain in the effluent, thus increasing the negative impacts on ecosystems. Carbaryl and malachite green, products used to control parasites, are agents that cause the reduction of bioluminescence of the marine bacteria *Vibrio fischeri* (Hernando *et al.* 2007). In addition, antibiotics used to maintain healthy organisms are responsible for affecting the growth, cellular volume and metabolic activity of the marine microalgae *Tetraselmis suecica* (Seoane *et al.* 2014).

Impurities present in the raw materials used in shrimp farming can contribute to the toxicity of the effluent. Such impurities are usually found in fertilizers, fishmeal, and feed. Lacerda *et al.* (2011) found a higher amount of mercury (Hg) in the particulate fraction of a shrimp farm effluent when compared with water uptake. The authors also found traces of mercury, fertilizer and lime in the feed, with the feed being the main responsible for the amounts of Hg released. Vaisman *et al.* (2005) also noted that Hg usually contaminates areas receiving aquaculture effluent discharges. Similarly, copper (Cu) is common in shrimp farm effluent. In a study in Northeastern Brazil, higher Cu amounts (13.1 to 79  $\mu\text{g g}^{-1}$ ) were found in the feed compared with other chemical additives and fertilizers used in the shrimp farm. All these situations contribute to Cu export to mangroves near shrimp farms, reaching 168 g Cu/ha in each shrimp farming cycle (Lacerda *et al.* 2006). These authors also observed increased copper concentrations in particulate matter in the mangroves directly receiving the effluent from the shrimp farm.

Two parameters of the analyzed effluent were in disagreement with the Brazilian effluent discharge resolution. Orthophosphate, which corresponds only to the bioavailable fraction of phosphorus, exceeded the allowable value for total phosphorus and the effluent also proved to be toxic, thus not complying with the legislation. Given that, shrimp farms should adopt practices such as constructed wetlands, in addition to the use of the sedimentation basin, to mitigate the orthophosphate levels and attenuate the toxicity of the produced effluent; otherwise, shrimp farm effluents may represent a risk to the bodies receiving these discharges.

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