**The effect of tilapia *Oreochromis niloticus* addition on the sediment of brackish low-salinity ponds to white shrimp *Penaeus vannamei* farming system during the wet and dry season**

**ABSTRACT.** The present study was carried out to determine the effect of tilapia on the physical-chemical and nutrient sediment parameters on an earthen pond in monoculture (shrimp) and co-culture (tilapia-shrimp) during the dry and wet seasons in a commercial farm during the period from mid-September to end-December 2017 and February to mid-May 2018. Chemical and physical analyses were realized on sediment samples from brackish water ponds representing shrimp farms in San Blas Nayarit Mexico. Concentrations of organic carbon, nitrogen, potassium, magnesium, and calcium were greater in co-culture ponds, and there was a tendency to a higher concentration of phosphorus and sodium in the sediment of monoculture ponds. The texture of the ponds showed a tendency in the dry season to increase the percentage of clay in co-culture due to the effect of bioturbation caused by tilapia. There was no evidence of poor development of sediment quality in any treatment. It was found that tilapia was a factor that affected sediment quality, differences in growth between shrimp monoculture and tilapia-shrimp co-culture can be explained, at least in part, by differences in their sediment.

**Keywords:** *Oreochromis niloticus*; *Litopenaeus vannamei*;sediment; co-culture; brackish pond

**INTRODUCTION**

The addition of tilapia to shrimp ponds has been used to improve water and sediment quality and shrimp production, compared to shrimp monoculture (Perschbacher & Stickney, 2017). Tilapia has been cultivated with a variety of fish and crustaceans under co-culture conditions mainly to improve production and have better environmental control of the farming system (Milstein & Hernández, 2017). The co-culture of tilapia with white shrimp *Litopenaeus* *vannamei*, has been shown to improve production and nutrient utilization in relation to shrimp monoculture, obtaining a better profitability and less environmental contamination (Sun *et al*., 2011; Bessa *et al.,* 2012; Hernández-Barraza *et al*., 2013; López-Gómez *et al*., 2017; Fitzsimmons & Shahkar, 2017). It encourages the development of bacterial communities, fungi, algae, and bioflocs of the native biota of the shrimp where the co-culture is carried out (Fitzsimmons & Shahkar, 2017), as in brackish low-salinity ponds. In the last years the co-culture of tilapia-shrimp has had a greater boom in small commercial farms of shrimp culture *P. vannamei* in Latin America among which are Brazil, Peru, Ecuador, Honduras, and Mexico mainly, because it has been found that this culture strategy reduces diseases such as that caused by White Spot Shrimp Virus, *Vibrio harveyi* and *Vibrio parahaemolyticus* (Rodríguez-Grimón, 2003; Cruz *et al*., 2008; Tran *et al*., 2014). In the Northwest of Mexico, most shrimp farms use the semi-intensive system in earthen ponds, where the southern Gulf of California farms are small 1 to 50 Has (Ponce-Palafox *et al*., 2011), where some follow the strategy of simultaneous polyculture system (tilapia and shrimp are grown together in a pond), which is common in other parts of the world (Fitzsimmons & Shahkar, 2017).

Studies on tilapia-shrimp co-culture have been carried focused on the effect upon water quality (Alam *et al*., 2008; Brito *et al*., 2017), feeding (Jatobá e*t al*., 2011), density (Candido *et al*., 2005), growth performance (Hernández-Barraza *et al*., 2012), production (Wang & Lu, 2016), diseases (Dash *et al*., 2017), and profitability (Muangkeow *et al*., 2007). However, studies on the effects on soil-sediment are scarce. Although, it has been determined that conditions at the bottom of the pond have a high influence, on water quality and the production of shrimp (Sonnenholzner & Boyd, 2000).

In the sediment shrimp spend the majority of the culture at the water-sediment interface, so a change in the sediment has direct effects on the shrimp (FAO, 2013), as well as the risk of contracting a bacterial disease (Nimrat *et al*., 2008). The conditions of sediment and water quality in shrimp farming earthen ponds determine their health and the success of the culture (Saraswathy *et al.,* 2019). In this type of pond sediment is formed on the ground composed mainly of higher concentration of organic carbon originated from plankton, feces, balanced feed and fertilizer (Munsiri *et al.,* 1995). What causes the nitrogen and phosphorus compounds are a higher concentration in the sediment than in the water column (Yang *et al.,* 2019). It has been found that within the first 4 cm depth is the zone of the sediment where nitrogen and phosphorus are released and transported (Cheng *et al*., 2014) and where the effect of tilapia of omnivores-detritivores habits on the sediment can be better detected. There are many studies on water and sediment quality of *Oreochromis niloticus* (Ram *et al*., 1982; Avnimelec *et al*., 1999; Adámek & Maršálek 2013; Fan *et al*., 2017; Omofunmi *et al*., 2018)and *L. vannamei* (Nimrat *et al*., 2008; Joyni *et al*., 2011; Van Duc *et al*., 2017; Yang *et al*., 2019) in ponds. However, there are few studies on the effect of tilapia-shrimp co-culture in rustic ponds at different time of the year. Therefore, this study aimed to determine the effect of tilapia on the physical-chemical and nutrients soil parameters on an earthen pond in mono and co-culture with shrimp during the dry season (concentration conditions) and rainfall season (dilution conditions) in a commercial farm.

**MATERIALS AND METHODS**

**Study area**

The experiment was carried out at Chiripa, San Blas Nayarit, Northwest coast of Mexico (21°37'34.53"N, 105°18'16.31"W). The mean temperature of the environment was from 22-26°C (García, 2004). The earthen ponds have a surface area from 2.75 to 3.2 ha and 1.2 m deep. The ponds use water from a canal with salinity from 8.5 to 18.4 ppt, depending on the time of year.

**Experimental animals**

Post-larval (PL15) Pacific white shrimp (*Penaeus vannamei*) mean initial weight = 0.001 ± 0.001 g were bought from a commercial hatchery, located in the Bay of Matanchen, municipality of San Blas, Nayarit, called "Acuacultura Integral, S.A. de CV." All the tilapia (5.0 ± 0.6 g) were transported from fingerling commercial hatchery "Genetilapia SA de CV," located at Rosario Sinaloa, Mexico.

**Experimental design**

Two trials were carried out using a completely randomized design consisting of two treatments (wet and dry season) with three replications (ponds) for each. The first trial takes106 days of mid-September to end-December 2017 (wet season) and the second trial took the same time as the first and was of February to mid-May 2018 (dry season). Treatments were shrimp monoculture weat season (SMW) and dry season (SMD) and tilapia-shrimp co-culture weat season (TSW) and dry season (TSD). Shrimp were stocked (10 org m-2) in monoculture ponds for all treatments; tilapia (4 org m-2) and shrimp (10 org m-2) were stocked in co-culture ponds. First, the shrimp were stocked and then the tilapia was added seven days later directly in each of the ponds, in all cases. The shrimp and tilapia in each earthen pond were collected from a harvesting pit. The tilapia and shrimp were fed four times a day (07:00, 11:00, 15:00 and 19:00 h) commercial pellets for shrimp feed brand “Paymar" with 40-20% (initial-final) and 10-20% (initial-final) protein and lipids, respectively were used. The ponds were initially fertilized ten days before shrimp and tilapia stocking with NutriLake-P® commercial fertilizer (5 kg ha-1 in each pond) for growth of natural food organisms.

**Sediment analysis**

A kilogram of soil samples was collected from each tank at the initial and at the end of the culture. In each pond, salinity (potential meter) and pH (Jackson, 1973, method) were determined. Organic matter (OM) was measured according to the procedures described in Rayment & Higginson (1992). Kjeldahl Nitrogen was measured to determine total organic nitrogen (TN) in the soil according to the procedure described in USDA (2014). Total phosphorus (TP) was measured in soil extract obtained from perchloric digestion (Horwitz, 2002). Macronutrients such as calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K) were analyzed by extraction with ammonium acetate pH = 7 modified (Woerner, 1989). A granulometry study was carried out according to Soil Survey Staff (1999) sieving the sediment sample and Pipette method (Day, 1965) was used to determine the following fractions clay (CL) (<2 μm), silt (SI) (2-50 μm), and sand (SA) (50-2000 μm). Gravel fractions (> 2 mm) were not detected. Other analyses were carried out on the fine fractions of the sediment (<2 mm) following the method used by Aldama-Rojas *et al*. (2011).

**Statistical analysis**

The results of sediment variables were checked using Levene's test for homogeneity of variances and Shapiro-Wilk's test for normality (Sokal & Rolhf, 2012). Differences between treatments were compared using one-way analysis of variance (ANOVA), and Tukey’s test was applied to determine which treatments differed significantly. To determine the most important sediment parameters and the multivariate effects on the sampling sites, a Correlation matrix, Principal Components, and Discriminant analysis were performed. Data were analyzed using Statistica for Windows (version 5.5 Inc., USA).

**RESULTS**

In the wet season, no significant differences (*P* > 0.05) in pH were found between all sampling stations (Table 1). There were significant differences (*P* < 0.05) between the initial concentrations of OM, TN, TP, Mg, and Na and those recorded at the end of the culture. The OM, TP, Mg, Ca, Na, SA, SI, and CL concentrations were similar in the monoculture and co-culture system at the end of the experiment. Only significant differences (*P* < 0.05) were found in TN and K in the two strategies used, higher TN concentration and lower K in co-culture.

**Table 1.** Sediment variables in monoculture (shrimp) and co-culture system (tilapia/shrimp) in water indoor pond water (IPW) during the wet season for 106 days. Values in each row superscripted with different letters indicate significant differences between groups (*P* < 0.05).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter/Treatment | Initial sediment | | Final sediment | |
| SMW | TSW | SMW | TSW |
| OM (%) | 4.50 ± 2.1b | 5.30 ± 2.1b | 6.30 ± 1.8a | 8.70 ±1.6a |
| TN (kg ha-1) | 156.0 ± 42.2d | 312.0 ±42.3b | 275.0 ± 32.7c | 405.0 ± 32.9a |
| TP (mg kg-1) | 1,023.0 ± 345.1b | 1,215.0 ±341.2b | 1,653.7 ± 170.1a | 1,456.0 ± 173.2b |
| K (mg kg-1) | 32.1 ± 7.0a | 45.6 ± 13.5b | 39.4 ± 7.2b | 49.3 ± 13.9a |
| Mg (mg kg-1) | 1,786.0 ± 176.6b | 1,254.0 ± 252.4c | 2,036.0 ± 175.2 a | 2,038.0 ± 259.1 a |
| Ca (mg kg-1) | 1.60 ± 0.51b | 2.30 ± 0.31a | 2.40 ± 0.52a | 2.90 ± 0.29a |
| pH | 7.80 ± 0.14a | 7.50 ± 0.02a | 7.60 ± 0.16a | 7.60 ± 0.01a |
| Na (mg kg-1) | 1,456.0 ± 394.5b | 1,136.0±528.4b | 2,064.0 ± 391.2a | 2,039.0 ± 516.3a |
| SA (%) | 11.30 ± 0.27b | 14.50 ± 0.6a | 10.50 ± 0.29a | 10.50 ± 0.2a |
| SI (%) | 18.30 ± 7.1b | 32.10 ± 2.7a | 28.40 ± 7.3a | 28.40 ± 2.3a |
| CL (%) | 70.4 ± 6.7a | 53.4 ± 4.4c | 61.3 ± 6.1b | 61.1 ± 4.1b |

In the dry season, there were significant differences between the initial concentrations of OM, TN, K, Mg, Ca, pH, Na, SA, SI, and CL and those recorded at the end of the culture (Table 2).

**Table 2.** Sediment variables in monoculture (shrimp) and co-culture system (tilapia/shrimp) in water indoor pond water (IPW) during the dry season for 106 days. Values in each row superscripted with different letters indicate significant differences between groups (*P* < 0.05).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter/Treatment | Initial sediment | | Final sediment | |
| SMW | TSW | SMW | TSW |
| OM (%) | 5.70 ± 1.1b | 8.20 ± 1.1b | 10.00 ± 1.8a | 12.00 ±1.6a |
| TN (kg ha-1) | 260.0 ± 2.2b | 278.0 ±2.3b | 320.0 ± 2.7a | 346.0 ± 2.9a |
| TP (mg kg-1) | 940.0 ± 0.1b | 540.0 ±0.2 c | 1,255.2 ± 0.1a | 792.7 ± 0.2b |
| K (mg kg-1) | 20.64 ± 0.01b | 15.12 ± 0.01c | 29.92 ± 0.02a | 32.74 ± 0.01a |
| Mg (mg kg-1) | 2,423.0 ± 0.6b | 2,563.0 ± 2.4b | 3,292.0 ± 1.0a | 3,891.0 ± 1.1a |
| Ca (mg kg-1) | 1.42 ± 0.01b | 2.30 ± 0.01a | 2.10 ± 0.02a | 2.40 ± 0.01a |
| pH | 7.90 ± 0.01a | 8.10 ± 0.02a | 7.50 ± 0.06b | 7.30± 0.01b |
| Na (mg kg-1) | 3,036.0 ± 0.4b | 2,530.0 ± 0.4c | 5,632.0 ± 0.2a | 5,280.0 ± 0.3a |
| SA (%) | 19.3 ± 3.1b | 15.3 ± 0.7b | 24.9 ± 0.2b | 24.9 ± 0.2b |
| SI (%) | 35.3± 0.2a | 39.3± 0.6c | 67.3 ± 3.3a | 65.2 ± 0.3a |
| CL (%) | 45.4 ± 4.7a | 45.4 ± 2.4b | 7.8 ± 4.1c | 9.9 ± 2.1c |

The OM, TN, K, Mg, Ca, pH, Na, SA, SI and CL concentrations were similar in the monoculture and co-culture system at the end of the experiment. Only significant differences were found in TP in the two strategies used, higher TP concentration in monoculture. There was a tendency for the sediment of the co-culture system to have a higher percentage of clay.

In the wet season, the most significant proportional correlations were OM-TN, TP-pH-SI, K-Mg-Na-CL, Mg-Na and inversely proportional between SA-SI. (Table 3), and in the dry season, the most significant proportionally correlations were OM-TN, TP-pH, K-Mg, and pH-Na. (Table 4).

**Table 3.** Correlations matrix sediment parameters in monoculture (shrimp) and co-culture system (tilapia/shrimp) in all treatments during the wet season for 106 days. \*Marked correlations are significant at *P* < 0.05.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Variables | OM | TN | TP | K | MG | CA | PH | NA | SA | SI | CL |
| OM | 1 |  |  |  |  |  |  |  |  |  |  |
| TN | **0.77\*** | 1 |  |  |  |  |  |  |  |  |  |
| TP | 0.53 | 0.64 | 1 |  |  |  |  |  |  |  |  |
| K | 0.22 | -0.23 | -0.15 | 1 |  |  |  |  |  |  |  |
| MG | 0.34 | 0.21 | 0.47 | **0.72\*** | 1 |  |  |  |  |  |  |
| CA | -0.36 | -0.22 | -0.54 | 0.46 | 0.26 | 1 |  |  |  |  |  |
| pH | 0.5 | 0.33 | **0.75\*** | 0.23 | 0.53 | -0.29 | 1 |  |  |  |  |
| NA | 0.26 | 0.06 | 0.09 | **0.77\*** | **0.72\*** | 0.58 | 0.56 | 1 |  |  |  |
| SA | -0.33 | -0.27 | -0.57 | -0.21 | -0.59 | 0.38 | -0.14 | 0.12 | 1 |  |  |
| SI | 0.57 | 0.67 | **0.87\*** | -0.15 | 0.44 | -0.58 | 0.39 | -0.17 | **-0.84\*** | 1 |  |
| CL | 0.22 | -0.44 | -0.24 | **0.77\*** | 0.29 | -0.05 | 0.16 | 0.34 | -0.15 | -0.2 | 1 |

**Table 4.** Correlations matrix sediment parameters in monoculture (shrimp) and co-culture system (tilapia/shrimp) in all treatments during the dry season for 106 days. \*Marked correlations are significant at *P* < 0.05

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Variables | OM | TN | TP | K | MG | CA | PH | NA | SA | SI | CL |
| OM | 1.00 |  |  |  |  |  |  |  |  |  |  |
| TN | **0.77\*** | 1.00 |  |  |  |  |  |  |  |  |  |
| TP | 0.53 | 0.64 | 1.00 |  |  |  |  |  |  |  |  |
| K | 0.22 | -0.23 | -0.15 | 1.00 |  |  |  |  |  |  |  |
| MG | 0.34 | 0.21 | 0.47 | **0.72\*** | 1.00 |  |  |  |  |  |  |
| CA | -0.36 | -0.22 | -0.54 | 0.46 | 0.26 | 1.00 |  |  |  |  |  |
| pH | 0.50 | 0.33 | **0.75\*** | 0.23 | 0.53 | -0.29 | 1.00 |  |  |  |  |
| NA | 0.43 | 0.51 | 0.78 | 0.17 | 0.65 | 0.00 | **0.89\*** | 1.00 |  |  |  |
| SA | -0.33 | -0.27 | -0.57 | -0.21 | -0.59 | 0.38 | -0.14 | -0.16 | 1.00 |  |  |
| SI | 0.57 | 0.67 | **0.87\*** | -0.15 | 0.44 | -0.58 | 0.39 | 0.45 | **-0.84\*** | 1.00 |  |
| CL | 0.22 | -0.44 | -0.24 | **0.77\*** | 0.29 | -0.05 | 0.16 | -0.17 | -0.15 | -0.20 | 1.00 |

Two groups of sediment variables were found, one related to the dynamics of phosphorus and nitrogen, and the other to changes in sediment composition with OM, K, Ca and pH through cluster analysis (Fig. 1a). The analysis of main components showed that four processes occur in the sediment, such as the SA-SI, TN-OM, pH and TP relationship (Fig. b).

The cluster analysis of the sampling stations showed that there is a significant difference (*P* < 0.05) between the shrimp monoculture ponds and those of tilapia-shrimp co-culture when forming the two groups (Fig. 2).

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**Figure 1.** Sediment variables of monoculture and co-culture (tilapia/shrimp) of all treatments, during the two production cycles in the year. a). Dendrogram from hierarchical agglomerative cluster analysis; b) Principal components analysis.

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**Figure 2.** Sediment sampling stations of monoculture and co-culture (tilapia-shrimp) of all treatments, during the rain and dry season. Dendrogram from hierarchical agglomerative cluster analysis.

**DISCUSSION**

To determine the effect of tilapia on the sediment of the shrimp ponds, the main nutrients (TN, TP and K), minor nutrients (Ca, Mg and Na) and the texture (SA, SI and CL) of the sediment at the bottom of shrimp monoculture and tilapia-shrimp co-culture were considered. Because it is trapping in the sediment of the ponds decreases the total productivity of the pond (Gul *et al.,* 2015). Concerning the sediment texture of the ponds (higher proportion of silt and clay) the analysis showed that the ponds are more than ten years old and are of the clay-loam type (Siddique *et al*., 2012). A high percentage of clay was found in the wet season due to the contribution of terrigenous material that drains the channel that supplies the water to the ponds. It showed a tendency in the dry season to increase the percentage of clay in co-culture due possibly to the effect of bioturbation caused by tilapia (Adámek & Maršálek 2013), although it is not significant. The pH registered in the ponds is close to that which is adequate for a good sediment production and in values that correspond to ponds with more than ten years of operation (Siddique *et al*., 2012; Gul *et al*., 2015).

In general, it was found that the concentration of main nutrients and minor nutrients in the sediment of shrimp ponds was increased during the production cycle, a process reported by Ritvo *et al.* (1998). The concentration of OM found in the sediment of all the ponds was within what was reported for shrimp ponds in brackish water areas (Smith, 1996). A greater amount of organic matter was present in the sediment of the co-culture due to the greater contribution of food and higher density of organisms concerning monoculture because the accumulation of sediments is mainly formed by exogenous contributions, eroded soil, uneaten food, feces, dead plankton and microorganisms (Hopkins *et al*., 1994). A high amount of TP was absorbed by the sediment of the monoculture ponds at the two seasons of the year, with more in the wet.

In comparison with the TN, what is in agreement with what was found for nitrogen and phosphorus in the soil of shrimp ponds (Paez-Osuna *et al.,* 1999; Martin *et al*., 1998). The greater differences found between the concentrations of TN and TP in the sediment of the ponds in comparison with the other nutrients studied is due to the effect of the tilapia in the co-culture on the sediment mediate the bioturbation process as it has been described by Adámek & Marsálek (2013). The present investigation corroborates that the effect of the tilapia, by the process of bioturbation of the sediment, affects the concentration of nitrogen and phosphorus. It increases the concentration of oxygen in the sediment (Brönmark & Hansson, 2005), improving the process of mineralization (Hansen *et al*., 1998) of the culture pond contributing to better growth of the shrimp as has been demonstrated in this work.

Potassium under culture conditions was supplied in ponds by fertilizer, balanced feed, tilapia, shrimp, water inflow and precipitation (Boyd *et al.,* 2007). In the co-culture, a higher concentration of K was recorded in the sediment compared to the initial, and there was a higher absorption in the co-culture sediment. However, harvesting and scraping after the production cycle decline significantly because it was removed through the discharged water, organisms and sediment removed from the pond (Boyd *et al*., 2007). Mg, Ca and Na was related to the enrichment of the clays fraction, because the smaller particles, particularly silts and clays, tend to adsorb and transport large amounts of nutrients due to their relatively larger specific surfaces. Also, it had a non-significant tendency to greater absorption of the co-culture sediment (Boyd *et al*., 1994).

In conclusion, in this experiment, tilapia was a factor that affected sediment quality, differences in growth between shrimp monoculture and tilapia-shrimp co-culture can be explained, at least in part, by differences in their sediment.

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