



RESEARCH ARTICLE

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Effects of white shrimp (*Litopenaeus vannamei*) and tilapia nilotica (*Oreochromis niloticus* var. Spring) in monoculture and co-culture systems on water quality variables and production in brackish low-salinity water earthen ponds during rainy and dry seasons

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Abstract

Aim of study: To determine the effects of white shrimp (*Litopenaeus vannamei*) and tilapia nilotica (*Oreochromis niloticus* var. Spring) in monoculture and co-culture on water quality variables and production in earthen ponds during dry and rainy seasons.

Area of study: A shrimp farm (total area 20 ha) at Chiripa, San Blas Nayarit, Northwest coast of Mexico (21° 37' 34.53" N; 105° 18' 16.31" W).

Material and methods: Two production cycles were performed in a completely randomized design consisting of two treatments and three replications each during rainy season (September-December) and dry season (February-May). Shrimp was the main crop and tilapia the secondary species.

Main results: White shrimp (10 org/m²) can be co-cultured with Nile tilapia at a stocking high density (4 org/m²), leading to improved water quality and better utilization of nutrients in dry season than in rainy season. However, the shrimp' highest weight was recorded in the tilapia-shrimp co-culture ponds during rainy season due to higher water temperature and better quality of live food. The mean individual weight, biomass and survival of the shrimp, in co-culture ponds were greater than those of the shrimp monoculture, in the two seasons studied.

Research highlights: There was a trend towards greater concentration of nutrients in the water of the monoculture ponds and, lower dissolved oxygen and higher BOD₅ in co-culture ponds in the dry season than in the rainy season.

Additional keywords: effects water; aquaculture; commercial farm; season different; polyculture.

Abbreviations used: DO (dissolved oxygen); FGA (specific growth rate); SGR (specific growth rate); TAN (total ammonia nitrogen); TN (total nitrogen); TP (total phosphorus); TSS (total suspended solids); CaCO₃ (alkalinity); BOD₅ (biochemical oxygen demand in 5 days).

Authors' contributions: All authors contributed equally to this work (conception; acquisition, analysis, interpretation of data; drafting of the manuscript; critical revision of the manuscript and statistical analysis).

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Introduction

In the last decade, bacterial and viral diseases have affected white shrimp production in several countries of Latin America. This has made some shrimp farmers to use polyculture or crop rotation for tilapia production as an alternative production system (Watanabe *et al.*,

2002). The polyculture or co-culture of shrimp-tilapia (Fitzsimmons & Shahkar, 2017) has been implemented in many countries for production and the efficiency of this culture strategy has been studied in recent years (Martínez-Porchas *et al.*, 2010; Yuan *et al.*, 2010; Shahin *et al.*, 2011; Bessa Junior *et al.*, 2012). This strategy has been used in commercial production systems because

it improves water quality, controls the growth of phytoplankton, reduces organic matter in effluents and controls outbreaks of diseases (bacteria and viruses) in both tilapia and shrimp (Yi & Fitzsimmons, 2004; Ye *et al.*, 2011). In Latin American countries such as Brazil, Honduras, and Mexico, tilapia are cultured in brackish ponds traditionally used only for shrimp farming (Alceste *et al.*, 2001; Wang & Lu, 2016). The culture of shrimp and tilapia, contributes to improve shrimp' health, production and increases profits in a co-culture system (Li & Dong, 2002; Hernandez-Barraza *et al.*, 2012). Tilapias are omnivorous and are mainly filter feeders, so they improve the quality of water in ponds (Diana *et al.*, 1991; Ruan *et al.*, 1992, 1993; Zhang *et al.*, 1999; Tian *et al.*, 2001a). In co-culture when shrimp die or are moribund, the tilapias consume those shrimp, limiting cannibalism and mode of disease transmission. Tilapia also contributes to a decrease in the number of pathogenic vectors by consuming small crustaceans and promoting the increase of Gram-positive bacteria (Watanabe *et al.*, 2002). Studies on ponds used for co-culturing of tilapia-shrimp have shown that with this method the concentration of the bacterium *Vibrio harveyi* that causes luminous bacterial disease is controlled, there is a sustainable stable environment for plankton and improvement in shrimp' survival (Cruz *et al.*, 2008). In the co-culture of tilapia-shrimp, different aspects such as productivity, feeding, density, time of culture and age of different species, combination of species and culture model have been investigated (Wang *et al.*, 1998; Hernandez-Barraza *et al.*, 2012; Bessa Junior *et al.*, 2012; Lopez-Gomez *et al.*, 2017).

In the monoculture of shrimp, the excess nutrients increase phytoplankton, ammonia concentrations, and modify oxygen dynamics (Midlen & Redding, 1998). By incorporating tilapia into the co-culture system, the

performance of the shrimp is increased by improving water quality (49.5%), reducing nutrients in effluents (22.6%) and reducing disease outbreak (11.8%) (Yi & Fitzsimmons, 2004; Lopez-Gomez *et al.*, 2017). Most of the recent studies on tilapia-shrimp co-culture are done indoors in concrete or synthetic ponds, with some of the physical-chemical variables being controlled (Hernandez-Barraza *et al.*, 2012; Simão *et al.*, 2013; Lopez-Gomez *et al.*, 2017; Sharawy *et al.*, 2017). However, for those done using rustic ponds under natural conditions in commercial farms, quantitative knowledge of the effect of this cultivation strategy on the aquatic environment is still required to better understand the underlying mechanisms of the dynamics of water quality. The aim of this work is to determine the effects of white shrimp (*Litopenaeus vannamei*) and tilapia nilotica (*Oreochromis niloticus* var. Spring) in monoculture and co-culture on water quality variables and production in brackish low-salinity water earthen ponds during dry and rainy seasons.

Material and methods

Study area

The experiment was carried out in a shrimp farm (total area 20 ha) at Chiripa, San Blas Nayarit, Northwest coast of Mexico (21° 37' 34.53" N; 105° 18' 16.31" W). It has a climate of type Aw; it is rainy in summer, September being the highest rainy month (Fig. 1); it has an average temperature of 22 to 26 °C (García, 2004). In the experimental period, a tropical storm (Pilar name) occurred at the end of September which caused alterations atypical to the hydrological system in the study area. The earthen

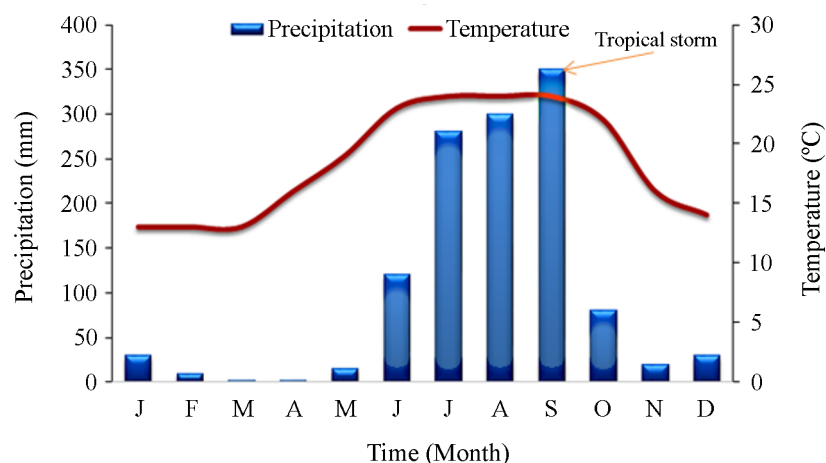


Figure 1. Mean temperature and precipitation of January to December 2017. Farm site data in San Blas, Nayarit, Mexico (García, 2004).

ponds have a mean surface area of 2.97 ha and their size range is from 2.75 to 3.2 ha, with a mean depth of 1.2 m. The farm uses water from an adjacent canal that acts as a reservoir. In this channel-reservoir comes a mixture of water with a salinity of 8.5 to 18.4 g/L; it originates from the mixture of estuary water called the Indian from Pericos Lagoon, with mean salinity of 38 g/L and freshwater of the Chacalilla stream that descends from the Huicicila-San Blas sub-basin. The farm pumps water from the reservoir channel into the ponds through several 10 Hp centrifugal pumps, passing through 50 μ screens to prevent predators and competitors. Water loss was compensated for weekly and replaced with 30% during the rainy season and 36% during the dry season. In addition, Paddle Wheel Aerator of 1 HP/750w was used at a ratio of 1 aerator per 1.5 ha.

Experimental animals

Post-larval (PL₁₅) Pacific white shrimp (*Litopenaeus vannamei*) were bought from a commercial hatchery, located in the Bay of Matanchen, municipality of San Blas, Nayarit, called 'Acuacultura Integral, S.A. de CV'. The shrimp were acclimated in the hatchery at 10 to 16 g/L salinity as was the initial condition of the production cycle. All the tilapia (5.0 \pm 0.6 g) were transported from fingerling commercial hatchery 'Genetilapia SA de CV', located at Rosario Sinaloa, Mexico. In the hatchery, the tilapia was acclimatized from fresh water to 10-15 g/L of salinity according to the production cycle.

Experimental design

Two trials (rainy and dry season) were conducted using a completely randomized design consisting of two treatments with three replications for each. The first trial lasted for 106 days (mid-September to end-December 2017; rainy season) and the second trial lasted for a period of 106 days (February to mid-May 2018; dry season). Treatments were shrimp monoculture rainy (SMR) and shrimp monoculture dry (SMD); tilapia-shrimp rainy (TSR) and tilapia-shrimp dry (TSD). Shrimp were stocked (10 org/m²), and Nile tilapia (4 org/m²) and shrimp (10 org/m²) were stocked in co-culture ponds (TSR and TSD). First, the shrimp were stocked and then the tilapia was added 7 days later directly in each of the ponds, in all cases. The shrimp-tilapia ratio was 1:0.4. At the end of the experiment, the ponds were drained with siphon pipes. The shrimp and tilapia in each earthen pond were collected from a harvesting pit.

Feed and feeding

The tilapia and shrimp were fed four times a day (07:00, 11:00, 15:00 and 19:00 h) at the beginning of the experimental period and 2 times a day (08:00 and 18:00 h) at the end of the culture. Commercial pellets for shrimp feed brand 'Paymar del Pacífico' with 40-25% (initial-final) and 10-20% (initial-final) protein and lipids, respectively were used. For the tilapia, Malta-Clayton was used with 35-25% (initial-final) and 15-20% (initial-final) protein and lipids, respectively. Feeding tray was used for feed adjustment according to the organisms' consumption. Feeding rate for tilapia-shrimp was between 20-2.8% and 16-2.6% of body weight per day, respectively. The ponds were initially fertilized 10 days prior to shrimp and tilapia stocking with NutriLake-P® commercial fertilizer (5 kg/ha in each pond) for growth of natural food organisms.

Water quality

Temperature, dissolved oxygen (DO), salinity, and pH were obtained *in situ* in all treatments between 08:00 and 9:00 am at medium depth daily, with a portable YSI-2030 Multiparameter (Yellow, Springs, OH, USA) and pH Hanna Model HI98190 (Rumani). Samples were taken using plastic bottles at the water inlet of the ponds, to the center of the ponds and water discharge sites for each of them. Total nitrogen (TN), nitrites (NO₂-N), nitrates (NO₃-N), total ammonium nitrogen (NH₃-N + NH₄⁺-N; TAN) and phosphates (PO₄-P), total phosphorus (TP), alkalinity (CaCO₃) were determined every 15 days using a YSI-9500 photometer (Yellow, Springs, OH, USA). Biochemical oxygen demand in 5 days (BOD₅) and total suspended solids (TSS) were measured following the procedures in the standard methods for the examination of water and wastewater (APHA, AWWA, WPCF, 1998), every 15 days.

Growth performance

During the experiment, samples were taken every 15 days, between 8:00 and 10:00 a.m. Fish and shrimp were caught with atarraya; 120 shrimp and 50 tilapias were weighed from each pond, every 2 weeks and returned immediately. Growth performance of the shrimp and tilapia was measured by initial weight, final weight, total weight gain, specific growth rate (SGR), consumed feed, feed conversion ratio (FCR), biomass and survival, according to López-Gómez *et al.* (2017). Final sampling was performed with 30% of the individuals from each pond.

Statistical analysis

Body weight of the organisms were expressed by mean \pm SD. Percent of data prior to the statistical analyses were arcsine-transformed. Water quality and growth performance variables were checked using Levene's test for homogeneity of variances and Shapiro-Wilk's test for normality (Sokal & Rolf, 1995). Differences between treatments were compared using one-way analysis of variance (ANOVA). If the main effects were significant, Tukey's test was applied to determine which treatments differed significantly. To determine the most important water variables 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 (vers. 5.5 Inc., USA).

Results







When comparing water quality variables by sampling site and season it was found that there was a significantly ($p < 0.05$) lower concentration of TP, alkalinity, BOD₅ and TSS in the inlet water than in the pond and outlet water and in nitrate, TN, BOD₅ and TSS during rainy (Table 1) and dry seasons (Table

2), respectively. Although there were no significant differences ($p > 0.05$), there was a tendency to have a higher concentration of TAN in the ponds and water outlet and a lower BOD₅ in the co-culture than in the monoculture ponds during rainy season (Table 1). During the dry season, the concentration of phosphates had a non-significant ($p > 0.05$) tendency to be more concentrated in the monoculture ponds.

The tropical storm that occurred in the month of September (Fig. 1) influenced the increase in the values and concentrations of temperature, salinity, TSS and alkalinity of the water in the ponds under study (Fig. 2), in October. There is a tendency to increase salinity and BOD₅ and to decrease the TSS from the start to the end of the culture cycle during the rainy season. In addition, there was a tendency to have a higher concentration of BOD₅, TSS and alkalinity in co-culture ponds than in monoculture ponds. In dry season, there was a tendency to increase the salinity and BOD₅ concentration towards the end of the culture and to decrease TSS concentration and DO from March to May (Fig. 3). There was a tendency over time to have a higher concentration of BOD₅, TSS and alkalinity in the co-culture ponds compared to the monoculture ponds.







In the rainy season, the highest correlation was directly proportional between the alkalinity with salinity and TSS (Table 3), and in the dry season,

Table 1. Water quality variables in monoculture (shrimp) and co-culture system (tilapia/shrimp) in water inlet channel, indoor pond water and water outlet channel during the rainy season for 106 days.

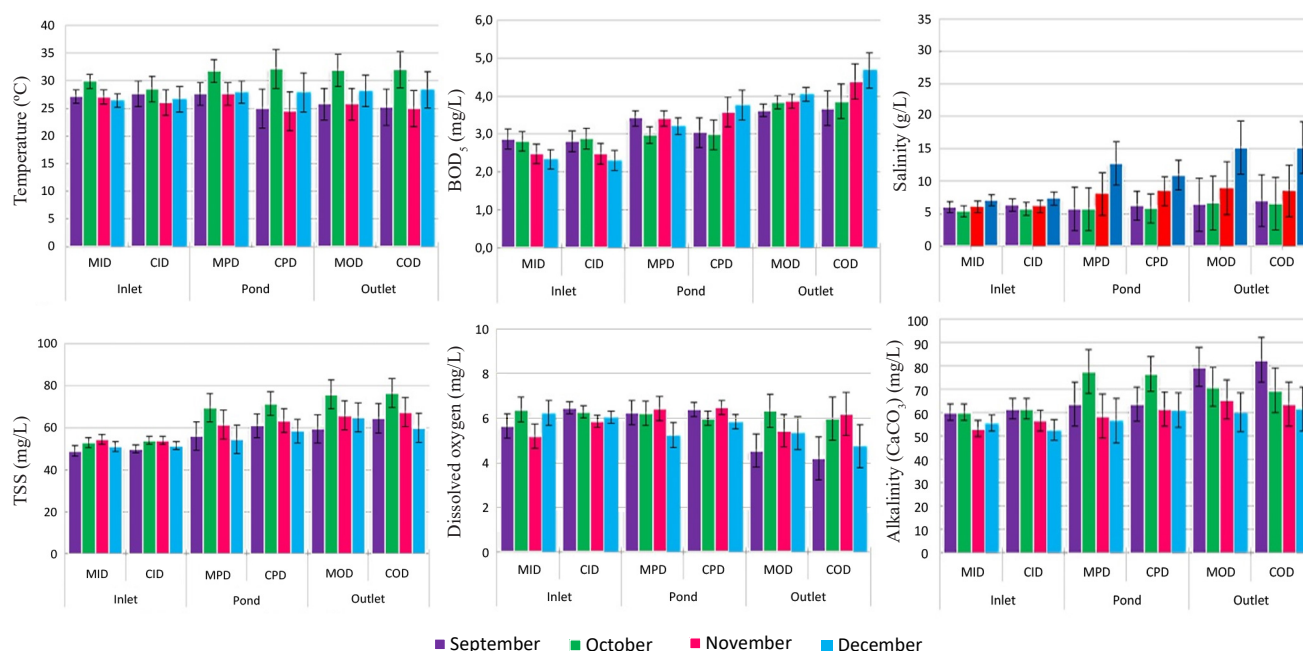
Variables	Water inlet channel		Indoor pond water		Water outlet channel	
	SMR	TSR	SMR	TSR	SMR	TSR
						
Temperature (C°)	28.5 \pm 2.3 ^a	28.6 \pm 3.3 ^a	29.4 \pm 1.7 ^a	29.2 \pm 3.3 ^a	29.2 \pm 3.4 ^a	29.2 \pm 3.8 ^a
Salinity (g/L)	8.5 \pm 3.7 ^a	8.6 \pm 4. ^a	9.9 \pm 3.9 ^a	9.7 \pm 4.6 ^a	11.0 \pm 4.3 ^a	11.2 \pm 4.5 ^a
pH	7.7 \pm 0.2 ^a	7.8 \pm 0.4 ^a	7.9 \pm 0.5 ^a	7.8 \pm 0.5 ^a	7.9 \pm 0.2 ^a	8.0 \pm 0.4 ^a
Dissolved oxygen (mg/L)	6.0 \pm 0.5 ^a	6.2 \pm 0.4 ^a	6.3 \pm 0.8 ^a	6.2 \pm 0.6 ^a	5.5 \pm 0.9 ^b	5.4 \pm 0.9 ^b
TAN (mg/L)	0.39 \pm 0.1 ^a	0.40 \pm 0.2 ^a	0.42 \pm 0.1 ^a	0.41 \pm 0.2 ^a	0.32 \pm 0.4 ^b	0.43 ^a \pm 0.2
NO ₂ -N (mg/L)	0.18 \pm 0.01 ^a	0.15 \pm 0.01 ^a	0.18 \pm 0.03 ^a	0.16 \pm 0.01 ^a	0.20 \pm 0.02 ^a	0.18 \pm 0.01 ^a
NO ₃ -N (mg/L)	1.2 \pm 0.1 ^a	1.3 \pm 0.2 ^a	1.6 \pm 0.1 ^a	1.5 \pm 0.2 ^a	1.5 \pm 0.1 ^a	1.4 \pm 0.1 ^a
TN (mg/L)	0.14 \pm 0.1 ^a	0.16 \pm 0.1 ^a	0.20 \pm 0.0 ^a	0.18 \pm 0.1 ^a	0.15 \pm 0.1 ^a	0.18 \pm 0.1 ^a
PO ₄ -P (mg/L)	0.03 \pm 0.1 ^a	0.04 \pm 0.6 ^a	0.04 \pm 1.1 ^a	0.03 \pm 0.04 ^a	0.05 \pm 0.09 ^a	0.04 \pm 0.09 ^a
TP (mg/L)	0.19 \pm 0.1 ^b	0.21 \pm 0.2 ^b	0.25 \pm 0.3 ^a	0.24 \pm 0.5 ^a	0.27 \pm 0.2 ^a	0.29 \pm 1.0 ^a
Alkalinity (CaCO ₃) (mg/L)	57.7 \pm 2.1 ^b	58.7 \pm 3.6 ^b	63.9 \pm 4.6 ^a	65.2 \pm 0.6 ^a	69.3 \pm 6.8 ^a	69.5 \pm 7.6 ^a
BOD ₅ (mg/L)	2.5 \pm 0.3 ^b	2.5 \pm 3.2 ^b	3.4 \pm 0.2 ^a	3.8 \pm 0.9 ^a	3.7 \pm 0.3 ^a	3.8 \pm 0.5 ^a
TSS (mg/L)	51.9 \pm 2.9 ^b	52.5 \pm 3.2 ^b	60 \pm 2.4 ^a	60.9 \pm 0.1 ^a	65.7 \pm 5.6 ^a	66.1 \pm 7.0 ^a
Color			Green	Brown		

SMR: shrimp monoculture rainy. TSR: tilapia-shrimp rainy. TAN: total ammonia nitrogen. NO₂-N: nitrite. NO₃-N: nitrate. TN: total nitrogen. PO₄-P: orthophosphates. TP: total phosphorus. BOD₅: biochemical oxygen demand in 5 days, TSS: total suspended solids. ^aMeans with different letter in the same row are significantly different ($p < 0.05$).

Table 2. Water quality variables in monoculture (shrimp) and co-culture system (tilapia/shrimp) in water inlet channel, indoor pond water and water outlet channel during the dry season for 106 days.

Variables	Water inlet channel		Indoor pond water		Water outlet channel	
	SMD	TSD	SMD	TSD	SMD	TSD
						
Temperature (C°)	27.3 ± 1.1 ^a	27.4 ± 1.1 ^a	27.8 ± 1.6 ^a	27.8 ± 1.8 ^a	27.8 ± 1.0 ^a	27.9 ± 1.2 ^a
Salinity (g/L)	14.4 ± 2.2 ^b	14.7 ± 2.3 ^b	16.3 ± 2.7 ^a	16.7 ± 2.9 ^a	17.8 ± 2.5 ^a	18.4 ± 3.4 ^a
pH	7.6 ± 0.1 ^a	7.6 ± 0.2 ^a	7.6 ± 0.1 ^a	7.6 ± 0.2 ^a	7.6 ± 0.2 ^a	7.6 ± 0.2 ^a
Dissolved oxygen (mg/L)	4.6 ± 0.6 ^a	4.7 ± 2.4 ^a	4.6 ± 1.0 ^a	4.4 ± 1.1 ^a	4.4 ± 0.8 ^a	4.5 ± 0.7 ^a
TAN (mg/L)	0.50 ± 0.01 ^a	0.50 ± 0.01 ^a	0.48 ± 0.02 ^a	0.45 ± 0.01 ^a	0.47 ± 0.02 ^a	0.44 ± 0.02 ^a
NO ₂ -N (mg/L)	0.23 ± 0.01 ^a	0.20 ± 0.02 ^a	0.24 ± 0.06 ^a	0.23 ± 0.01 ^a	0.25 ± 0.07 ^a	0.25 ± 0.02 ^a
NO ₃ -N (mg/L)	0.71 ± 0.04 ^b	0.71 ± 0.04 ^b	0.78 ± 0.02 ^a	0.77 ± 0.03 ^a	0.83 ± 0.04 ^a	0.82 ± 0.04 ^a
TN (mg/L)	0.26 ± 0.2 ^b	0.25 ± 0.6 ^b	0.32 ± 0.2 ^a	0.31 ± 0.2 ^a	0.32 ± 0.4 ^a	0.32 ± 0.3 ^a
PO ₄ -P (mg/L)	0.41 ± 0.1 ^a	0.45 ± 0.7 ^a	0.42 ± 0.3 ^a	0.37 ± 0.3 ^a	0.41 ± 0.3 ^a	0.38 ± 0.2 ^a
TP (mg/L)	0.29 ± 0.7 ^a	0.31 ± 0.4 ^a	0.35 ± 0.1 ^a	0.33 ± 0.1 ^a	0.36 ± 0.2 ^a	0.33 ± 0.1 ^a
Alkalinity (CaCO ₃) (mg/L)	59.9 ± 6.1 ^a	59.2 ± 0.1 ^a	59.8 ± 5.5 ^a	60.9 ± 4.9 ^a	60.4 ± 5.2 ^a	60.5 ± 4.5 ^a
BOD ₅ (mg/L)	2.7 ± 0.3 ^b	2.7 ± 0.5 ^b	3.0 ± 0.5 ^a	3.2 ± 0.6 ^a	3.3 ± 0.8 ^a	3.4 ± 0.8 ^a
SST (mg/L)	48.6 ± 2.9 ^b	49.4 ± 2.3 ^b	52.6 ± 3.3 ^a	52.4 ± 2.4 ^a	53.8 ± 3.8 ^a	53.7 ± 2.2 ^a
Color			Green	Brown		

SMD: shrimp monoculture dry. TSD: tilapia-shrimp dry. TAN: total ammonia nitrogen. NO₂-N: nitrite. NO₃-N: nitrate. TN: total nitrogen. PO₄-P: orthophosphates. TP: total phosphorus. BOD₅: biochemical oxygen demand in 5 days, TSS: total suspended solids. ^aMeans with different letter in the same row are significantly different ($p < 0.05$).

**Figure 2.** Fluctuation of temperature, salinity, dissolved oxygen, BOD₅, TSS and alkalinity throughout the experimental period of 106 days, during the rainy season. Error bars indicate SE. MIR: monoculture inlet rainy. CIR: co-culture inlet rainy. MPR: monoculture pond rainy. CPR: co-culture pond rainy. MOR: monoculture outlet rainy. COR: co-culture outlet rainy.

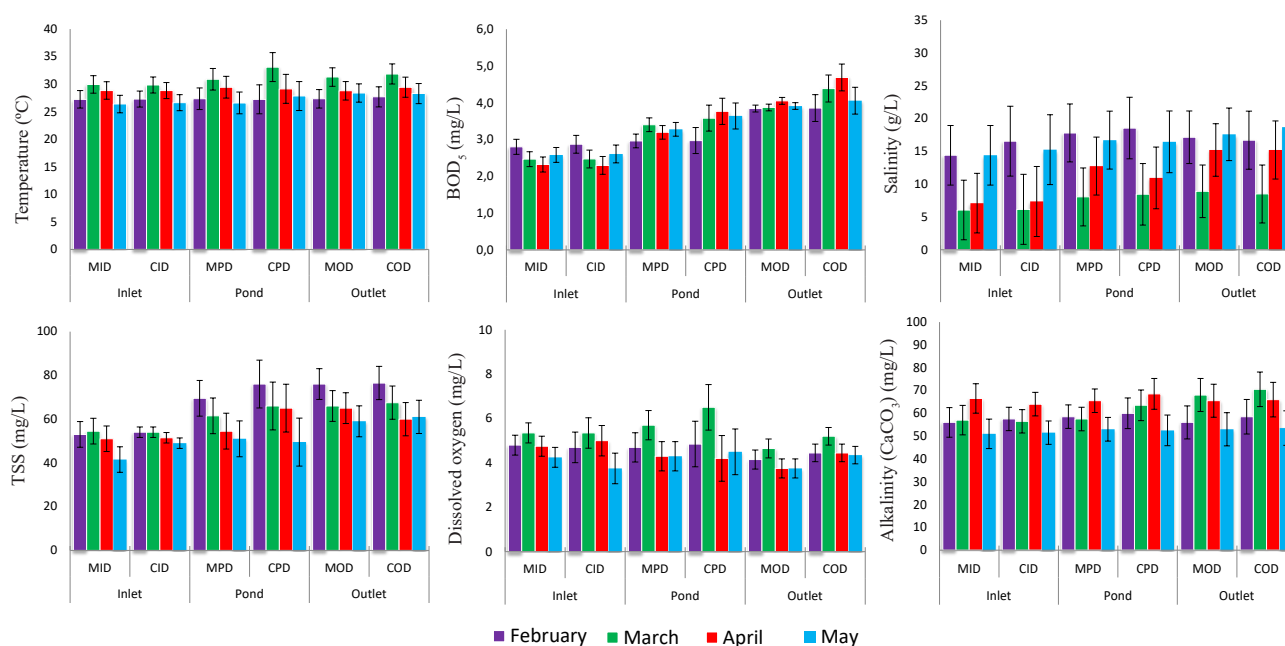


Figure 3. Fluctuation of temperature, salinity, dissolved oxygen, BOD₅, TSS and alkalinity throughout the experimental period of 106 days, during the dry season. Error bars indicate SE. MID: monoculture inlet dry. CID: co-culture inlet dry. MPD: monoculture pond dry. CPD: co-culture pond dry. MOD: monoculture outlet dry. COD: co-culture outlet dry.

it was between TSS with salinity and temperature (Table 4). In the dry season, the greatest number of significant correlations of the water quality variables was presented. Temperature correlated significantly ($p < 0.05$) with most of the studied variables. DO only had a significant correlation ($p < 0.05$) that is inversely proportional to temperature and salinity in the dry season.

Cluster analysis showed that there are two relations of variables such as the interaction of nutrients and the physico-chemical variables of the water in the system under study (Fig. 4a). Three groups were found between the variables that describe the behavior of the water quality in the system and the BOD₅ as determinants of the variability of the behavior of the water, through PC analysis (Fig. 4b). The first group showed the relation

Table 3. Correlations matrix water quality variables in monoculture (shrimp) and co-culture system (tilapia/shrimp) in all treatments during the rainy season for 106 days.

	Tem	pH	Sal	DO	TN	NO ₂	NO ₃	PO ₄	TP	Alk	TAN	BOD ₅	TSS
Tem	1												
pH	0.36	1											
Sal	0.71*	0.53	1										
DO	-0.27	-0.35	-0.34	1									
TN	0.55	0.37	0.45	0.06	1								
NO ₂	0.69*	0.14	0.51	-0.37	-0.05	1							
NO ₃	0.83*	0.28	0.68*	0.16	0.42	0.54	1						
PO ₄	0.15	0.06	0.45	0.17	0.24	0.17	0.27	1					
TP	0.56	0.63*	0.81*	-0.51	0.37	0.32	0.42	0.47	1				
Alk	0.73*	0.56	0.93*	-0.54	0.33	0.67*	0.56	0.43	0.83*	1			
TAN	0.43	0.18	0.2	0.01	0.76*	-0.18	0.33	-0.17	0.15	0.1	1		
BOD ₅	0.27	0.02	-0.16	0.22	0.37	-0.06	0.19	-0.12	-0.18	-0.08	0.53	1	
TSS	0.74*	0.50	0.81*	-0.62*	0.28	0.76*	0.56	0.32	0.74*	0.88*	0.06	-0.21	1

Tem: temperature. Sal: salinity. DO: dissolved oxygen. TN: total nitrogen. NO₂: nitrite. NO₃: nitrate. PO₄: orthophosphates. TP: total phosphorus. Alk: alkalinity. TAN: total ammonia nitrogen. BOD₅: biochemical oxygen demand in 5 days. TSS: total suspended solids. *Marked correlations are significant at $p < 0.05$.

Table 4. Correlations matrix water quality variables in monoculture (shrimp) and co-culture system (tilapia/shrimp) in all treatments during the dry season for 106 days.

	Tem	pH	Sal	DO	TN	NO ₂	NO ₃	PO ₄	TP	Alk	TAN	BOD ₅	TSS
Tem	1.00												
pH	0.13	1.00											
Sal	0.89*	0.03	1.00										
DO	-0.62*	0.09	-0.75*	1.00									
TN	0.65*	0.38	0.72*	-0.40	1.00								
NO ₂	0.84*	0.01	0.86*	-0.83*	0.48	1.00							
NO ₃	0.73*	0.22	0.91*	-0.59	0.81*	0.74*	1.00						
PO ₄	-0.73*	-0.19	-0.63*	0.33	-0.53	-0.65*	-0.51	1.00					
TP	0.80*	0.27	0.64*	-0.43	0.50	0.60	0.55	-0.37	1.00				
Alk	0.72*	0.36	0.60	-0.48	0.46	0.69*	0.52	-0.83*	0.54	1.00			
TAN	0.88*	0.21	0.75*	-0.45	0.79*	0.68*	0.68*	-0.68*	0.69*	0.68*	1.00		
BOD ₅	-0.77*	-0.06	-0.77*	0.26	-0.73*	-0.57	-0.75*	0.76*	-0.39	-0.54	-0.81*	1.00	
TSS	0.93*	0.00	0.94*	-0.65*	0.55	0.91*	0.82*	-0.73*	0.67*	0.70*	0.74*	-0.78*	1.00

Tem: temperature. Sal: salinity. DO: dissolved oxygen. TN: total nitrogen. NO₂: nitrite. NO₃: nitrate. PO₄: orthophosphates. TP: total phosphorus. Alk: alkalinity. TAN: total ammonia nitrogen. BOD₅: biochemical oxygen demand in 5 days. TSS: total suspended solids. *Marked correlations are significant at $p < 0.05$.

DO has with the oxidation of nutrients and the effect of its production on the pH. The second group showed the relation of the temperature with the process of dilution-concentration through the alkalinity and TSS variables. The interaction of the nutrients with the rainy and dry

seasons was determined by the salinity of the system water.

Cluster analysis classified the sampling stations, in the dry and rainy seasons (Fig. 5a) into two groups. The discriminant analysis confirmed the two groups

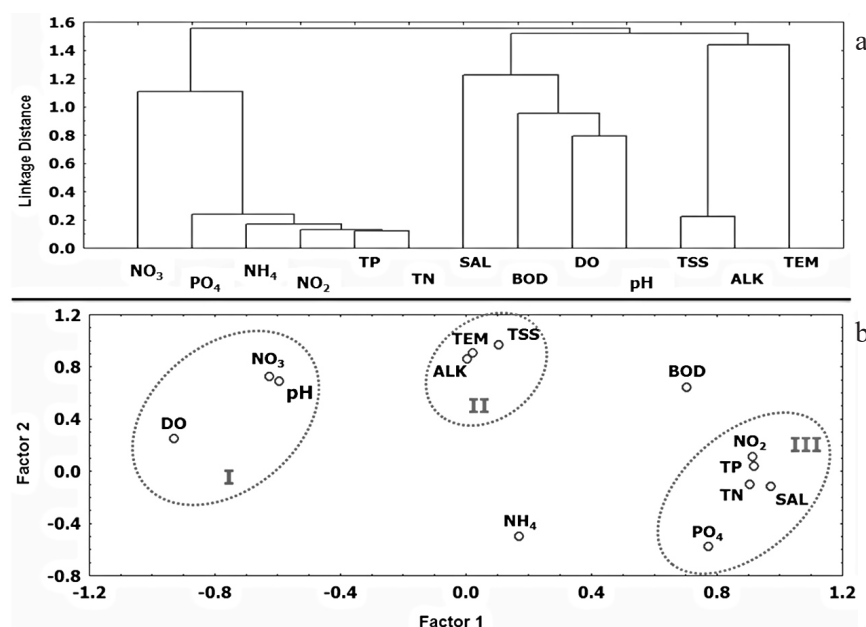


Figure 4. Water quality variables of monoculture and co-culture (tilapia/shrimp) of all treatments, during the two production cycles in the year. a) Dendrogram from hierarchical agglomerative cluster analysis; b) principal components analysis. TEM: temperature. SAL: salinity. DO: dissolved oxygen. TN: total nitrogen. NO₂: nitrite. NO₃: nitrate. PO₄: orthophosphates. TP: total phosphorus. ALK: alkalinity. TAN: total ammonia nitrogen. BOD₅: biochemical oxygen demand in 5 days. TSS: total suspended solids.

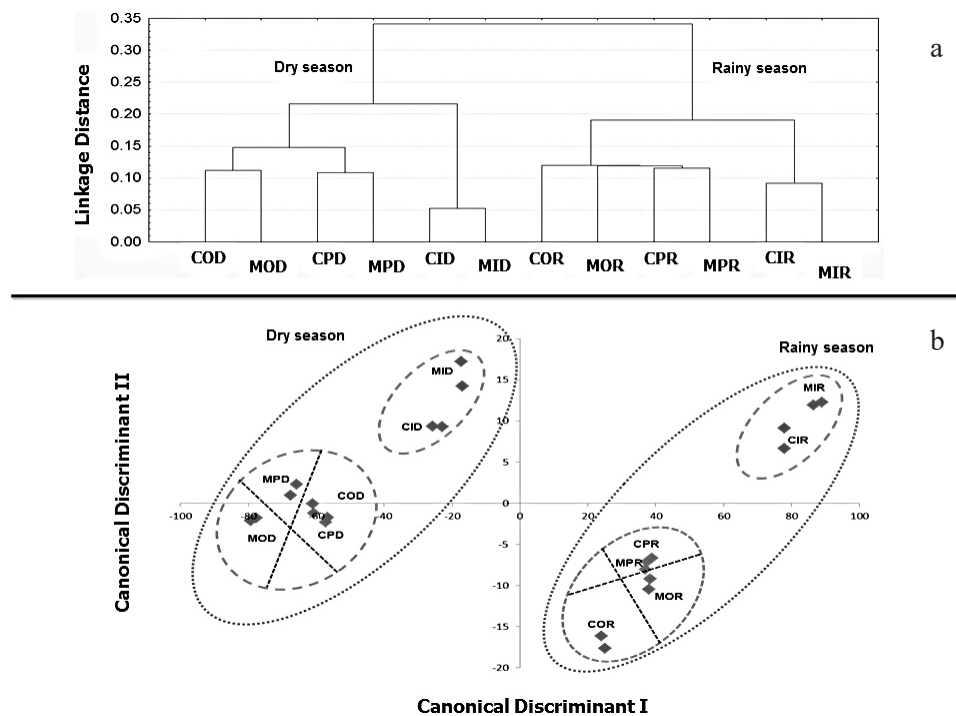


Figure 5. Water quality sampling stations of monoculture and co-culture (tilapia-shrimp) of all treatments, during the rainy and dry season. a) Dendrogram from hierarchical agglomerative cluster analysis; b) discriminant analysis. MIR: monoculture inlet rainy. CIR: co-culture inlet rainy. MPR: monoculture pond rainy. CPR: co-culture pond rainy. MOR: monoculture outlet rainy. COR: co-culture outlet rainy. MID: monoculture inlet dry. CID: co-culture inlet dry. MPD: monoculture pond dry. CPD: co-culture pond dry. MOD: monoculture outlet dry. COD: co-culture outlet dry.

due to the culture period. Within these two groups in both seasons of the year (Fig. 5b), there were sampling stations of the inlet in the first group and the other two, pond and outlet in the second group. In the sampling seasons of the dry period, there were differences in the water quality of the monoculture ponds and the co-culture pond, whereas in the rainy season there were no differences. The sampling stations of the water outlet of the monoculture ponds were different from all dry and rainy sampling stations.

The shrimp' final weight and biomass were significantly ($p < 0.05$) higher in the rainy season than in dry season (Table 5). FCR was significantly ($p < 0.05$) lower in co-culture than in monoculture and survival was significantly higher in co-culture than in monoculture. No significant ($p > 0.05$) differences were found between the treatments in initial weight, total weight gain and SGR. Food consumption and FCR were significantly ($p < 0.05$) lower in tilapia in the rainy season. All the other production variables did not have significant ($p > 0.05$) differences in the production periods. There was a non-significant tendency for tilapia to have greater weight and survival in the dry season. The co-culture had a significantly ($p < 0.05$) higher biomass production in the

rainy season than in the dry season and did not present significant differences in total weight gain, SGR and FCR. The discharge rates of TN and TP were higher in the monoculture ponds in all the treatments (Table 5). In the rainy season, the TP discharge rate was higher than in the dry season and the percentage, in general, was higher for nitrogen than for phosphorus.

Discussion

The variables of water quality during the two production cycles were within the appropriate intervals for the growth of tilapia and shrimp in monoculture and co-culture (Candido *et al.*, 2005). There was a higher concentration of oxygen in the rainy season than in the dry season and there was a non-significant tendency ($p > 0.05$) to present higher DO concentration and lower BOD_5 in shrimp monoculture than in co-culture. This was probably due to the combined effect of the two species (tilapia-shrimp), as reported for tilapia with other fish (Ibrahim & Naggar, 2010; Shoko *et al.*, 2014).

The concentration of nutrients in the water of the ponds did not present significant differences ($p < 0.05$)

Table 5. Performance of shrimp cultured for 106 days in monoculture and co-culture system, with tilapia nilotica and shrimp in earthen ponds during the rainy and dry season, and rate of discharge (%) of total nitrogen and total phosphorus.

Performance variables	Monoculture		Co-culture	
	Rainy	Dry	Rainy	Dry
Shrimp				
Initial weight (g)	0.6 ± 0.3 ^a	0.5 ± 0.2 ^a	0.6 ± 0.2 ^a	0.5 ± 0.2 ^a
Final weight (g)	14.8 ± 0.5 ^a	13.5 ± 0.3 ^b	15.1 ± 0.4 ^a	14.3 ± 0.2 ^b
Biomass (kg/pond)	3,291.5 ± 4.3 ^a	2,967.8 ± 3.7 ^a	3,382.6 ± 2.0 ^a	3,248.6 ± 3.0 ^a
Total weight gain (kg)	2,366.7 ± 15.0 ^a	2,600.0 ± 16.4 ^a	2,416.7 ± 11.9 ^a	2,760.0 ± 11.6 ^a
SGR (%/day)	3.0 ± 0.47 ^a	3.1 ± 0.60 ^a	3.0 ± 0.52 ^a	3.2 ± 0.54 ^a
Feed consumption (g/org)	2.3 ± 0.1 ^b	2.3 ± 0.2 ^a	1.7 ± 0.3 ^a	1.8 ± 0.1 ^a
FCA	2.45 ± 0.1 ^b	2.72 ± 0.2 ^b	2.23 ± 0.3 ^a	2.36 ± 0.1 ^a
Net yield (kg/ha)	1,028.6 ± 9.5 ^a	987.5 ± 7.2 ^a	1,057.1 ± 8.9 ^a	1,015.2 ± 7.1 ^a
Survival (%)	69.5 ± 5.4 ^b	68.7 ± 8.3 ^b	78.3 ± 6.4 ^a	75.2 ± 4.6 ^a
Tilapia				
Initial weight (g)			4.1 ± 0.2 ^a	4.2 ± 0.1 ^a
Final weight (g)			81.3 ± 2.8 ^a	86.7 ± 3.2 ^a
Biomass (kg/pond)			569.4 ± 0.3 ^a	592.8 ± 0.2 ^a
Total weight gain (kg)			172.2 ± 10.2 ^a	168.4 ± 9.6 ^a
SGR (%/day)			3.5 ± 0.1 ^a	3.2 ± 0.2 ^a
Feed consumption (g/org)			1.7 ± 0.1 ^b	2.4 ± 0.2 ^a
FCA			0.92 ± 0.2 ^a	1.12 ± 0.3 ^b
Net yield (kg/ha)			177.9 ± 5.2 ^a	185.3 ± 4.8 ^a
Survival (%)			87.6 ± 0.1 ^a	91.2 ± 0.2 ^a
Mix-culture				
Total biomass (kg/pond)			3,952.0 ^a	3,841.40 ^a
Total weight gain (kg/pond)			410.8 ^a	405.6 ^a
Specific growth rate (%/day)			3.2 ^a	3.3 ^a
Net yield (kg/ha)			1,235.0 ± 15.1 ^a	1,200.5 ± 14.6 ^a
Overall FCA			1.57 ^a	1.74 ^a
Discharged rates (%)				
TN	42.9	24.1	12.5	23.0
TP	31.6	20.7	14.3	8.5

SGR: specific growth rate. FCR: feed conversion ratio. TN: total nitrogen. TP: total phosphorus. ^aNumbers in a row with the same superscripts do not significantly differ.

in all the treatments. This has been found in most studies with mono and co-culture tilapia-shrimp (Tian *et al.*, 2001a; Alam *et al.*, 2008). However, there was a tendency to have lower TN and TP and their compounds had lower concentration in co-culture than in the monoculture as has been reported in other tilapia-shrimp co-culture studies (Tian *et al.*, 2001b). The average discharge rate of TN and TP was lower in the co-culture than in the monoculture and was lower for TN (21.9%) than the one recorded in co-culture in Southeast Asia (53.1%) using earthen ponds (Briggs

& Funge-Smith, 1994). Several processes contribute to a lower concentration of nutrients in co-culture, such as the accumulation and trapping of bound ammonia in the sediments, rapid uptake of ammonia by cyanobacteria and progressive grazing of tilapia on natural food (Chiu, 1988; Tendencia *et al.*, 2006; Yuan *et al.*, 2010). Regarding the coloration of the water in the ponds, it was found that in the tilapia-shrimp co-culture, in the first weeks green color appeared and later it remained brown until the end. On the other hand, the monoculture pond had green color throughout. This is

in agreement with the findings of Brito *et al.* (2017) who recorded that there was a low concentration of total phytoplankton (Chlorophytes, Cyanophytes, and Pyrrophyta) in monoculture and in co-culture there was greater Bacillariophyta mainly.

The density used in this work was within the optimal range of stocking reported for shrimp co-culture with tilapia (Wang *et al.*, 1998). The proportion of 10 shrimp/m² and 4 tilapias/m² used has been considered as a high density of tilapia in these systems (Thien *et al.*, 2004). It has also been used in other co-culture studies of shrimp (10 shrimp/m²), where there was good production and survival of *L. vannamei* (75.2 to 78.3%) and tilapia nilótica (87.6% to 91.2%) (Jatoba *et al.*, 2011; Simão *et al.*, 2013). Our study showed that tilapia up to 86.7 g can be co-cultured with shrimp without a decrease in growth or increase in FCR. Shrimp' FCR is lower in monoculture than in co-culture in indoors clear water systems (Hernández-Barraza *et al.*, 2013). However, in monoculture earthen ponds, it consumes more balanced feed and has higher FCR than in co-culture ponds. Shrimp and tilapia in rainy season consume less balanced feed and have lower FCR than in dry season. However, in monoculture earthen ponds, they consume more balanced feed and have higher FCR. In general, it has been found that the overall FCR is higher in clear water systems than in earthen ponds in monoculture and co-culture (Hernández-Barraza *et al.*, 2013).

Most studies on tilapia-shrimp interaction have been reported in indoors controlled systems in which total production of fish and shrimp was found to be higher in co-culture than in monoculture (Hernández-Barraza *et al.*, 2013); a similar behavior was recorded in earthen ponds as in our study. The greater weight and survival, and lower consumption of feed and FCA of the shrimp in the co-culture system can be attributed to the ecological role tilapia plays (Wang *et al.*, 1998). This is because it directly affects the following: decrease of phytoplankton predation pressure (Vinyard *et al.*, 1988), production of organic particles of balanced food, undigested food by tilapia and its fecal matter and indirect movement of water, activation of nitrogen and phosphorus cycle and the microbial biomass developed due to the bio-manipulation of tilapia (Gonzales-Corre, 1988; Ross *et al.*, 1988; Yi *et al.*, 2002; Cruz *et al.*, 2008; Apún-Molina *et al.*, 2015).

The higher survival rate found in adding tilapia to the shrimp culture has already been reported in other shrimp species (Akiyama & Anggawati, 1998). This was probably due to the inhibitory effect of tilapia on certain pathogenic microorganisms (Akiyama & Anggawati, 1998; Tendencia *et al.*, 2004). In the co-culture of tilapia-shrimp, survival was greater for

tilapia than for shrimp in our work, a relationship that has been found in most of the works of this same culture strategy (Simão *et al.*, 2013). There were no differences in the survival of tilapia in monoculture (87.6%) and co-culture (91.2%), which has been reported in other studies (Bessa Junior *et al.*, 2012) with the same species used in this study (84 to 100%). Natural earthen ponds with high quality and low salinity waters allow the sustainability of greater stocking density of tilapia (4 org/m²) than systems or ponds lacking substrate (1.2 org/m²) at the bottom (glass fiber or cement tanks). Natural systems maintain high rates of recovery of nutrients in the harvest and have less negative environmental impact (Yuan *et al.*, 2010).

Our results indicate that the addition of tilapia at high density (4 org/m²) into white shrimp culture (10 org/m²) can improve productivity, nutrient utilization, and environmental friendliness. The highest weight of shrimp was recorded in the tilapia-shrimp co-culture in the rainy season, due to higher water temperature and better quality of live food. There was a trend towards greater concentration of nutrients in the water of the monoculture ponds in the dry season and not in the rainy season. There was lower DO concentration in dry and higher BOD₅ in co-culture ponds. The average individual weight of the shrimp, biomass per pond and survival in the tilapia-shrimp co-culture in the two seasons studied were greater than those of the monoculture shrimp. In conditions of rural aquaculture sustainable in small farms, shrimp-tilapia co-culture should be promoted to improve the sustainability of shrimp culture in Mexico and Latin America. Finally, our results on the water quality of the systems under study showed that management strategies are required to improve the management of water in co-culture, such as the optimization of feeding frequency, management of trays, aeration system and the implementation of sequential bottom cleaning during the culture cycle.

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