

Research Article

Nitrogen dynamics model in zero water exchange, low salinity intensive ponds of white shrimp, *Litopenaeus vannamei*, at Colima, Mexico

F.A. Castillo-Soriano¹, V. Ibarra-Junquera¹, P. Escalante-Minakata¹,
O. Mendoza-Cano¹, J. de J. Ornelas-Paz², J.C. Almanza-Ramírez¹ & A.O. Meyer-Willerer¹

¹Bioengineering Laboratory, University of Colima, Km 9 carretera Colima
Coquimatlán, Coquimatlán, Col. 28400, México

²Centro de Investigación en Alimentación y Desarrollo A.C., Unidad Cuauhtémoc
Av. Río Conchos S/N, Parque Industrial, C.P. 31570, Cd. Cuauhtémoc, Chihuahua, México

ABSTRACT. We present a mathematical model based on differential equations describing the dynamics of nitrogen (NH_4^+ , NO_2^- , NO_3^- and organic nitrogen in phytoplankton) in ponds of white shrimp (*Litopenaeus vannamei*), with low salinity and zero turnovers, from planting to harvest. The model predicts the results of commercial production in three ponds. We show that this culture system, without replacement, retains the nitrogen and shrimp produced a lower feed conversion in comparison with systems with replacement. The model can be used to define strategies for improved performance.

Keywords: white shrimp, dynamical model, nitrogen, low salinity, Colima, Mexico.

Modelo de dinámica del nitrógeno en estanques de cultivo intensivo, con baja salinidad y sin recambio de agua, de camarón blanco, *Litopenaeus vannamei*, en Colima, México

RESUMEN. Se presenta un modelo matemático de la dinámica del nitrógeno (NH_4^+ , NO_2^- , NO_3^- y nitrógeno orgánico en fitoplancton) en estanques de camarón blanco (*Litopenaeus vannamei*) en condiciones de baja salinidad y sin recambio de agua. El modelo predice los resultados de producción comercial en tres estanques comerciales. Se demuestra que este sistema de cultivo, sin reemplazo, retiene el nitrógeno y los camarones producidos tienen una baja conversión de alimento en comparación con sistemas con recambio. El modelo se utiliza para evaluar mejores estrategias de manejo de estos ambientes.

Palabras clave: camarón blanco, modelo dinámico, nitrógeno, baja salinidad, Colima, México.

Corresponding author: Vrani Ibarra-Junquera (vij@ucol.mx)

INTRODUCTION

In traditional intensive shrimp cultures, pond water is frequently exchanged with a new external water supply in order to maintain desirable water quality for shrimp growth, avoiding nitrogen build up and oxygen depletion (Hopkins *et al.*, 1993). The production systems have been evolved from extensive to intensive with increasing inputs of high quality feed and water supply (Boyd, 1999) and a consequent increase in nutrient discharge. In Australia the water exchange (WE) ratio is of 5 to 10% daily (Burford & Lorenzen, 2004), in El Salvador it is as much as 25% (Lovel, 1988). In Mexico, the major producers in Sonora,

Sinaloa and Nayarit use a 5 to 15% daily WE ratio (Alonso-Rodriguez *et al.*, 2004). Such practices generate effluents typically enriched in suspended solids, nutrients, chlorophyll-*a*, and high biochemical oxygen demand (Paez-Osuna, 2001a, 2001b). Shrimp farming in conjunction with municipal and agricultural effluents can impact large ecoregions (Paez-Osuna *et al.*, 2003). Shrimp farming is facing criticisms for its unsustainable practices, which include their water management practices (Naylor *et al.*, 1998, 2000). A major concern associated with WE is diseases of viral origin. This problem has been associated with poor water quality intakes and also to the use of water coming from natural water bodies that

contain crustacean's natural populations (Kautsky *et al.*, 2000).

In contrast, in the state of Colima, located on the Mexican west coast, all shrimp farms produce at low salinity conditions, with zero water exchange ZWE, using paddle wheel aerators (Castillo-Soriano *et al.*, 2010). Colima produced 1,500 ton of shrimp in 2007 (Industria Acuicola, 2008) in 210 ha of 16 farms. The white shrimp (*Litopenaus vannamei*) is the species grown in farms located in the Coquimatlán and Tecomán regions. In Colima the intake water is used to fill the ponds initially and to maintain pond level lost by evaporation or filtration. Pond water is flushed of the pond spillway only at shrimp harvest. The farm's effluents enter the river systems, where they are used for agricultural purposes before finally arriving to coastal wetlands. In any crop the limiting factor is either the substrate least available relative to the requirement for the synthesis of the crop (Liebig, 1840) or in turn, the one that becomes toxic. Nitrogen (N) can be bought, as one of the key elements in aquatic environments and an important pond management variable. N input is sometimes applied in the form of fertilizers to enhance aquatic productivity, and always in the feed to directly enhance shrimp growth. Protein is feed's most expensive component (Thoman *et al.*, 2001) and N is not fully retained by shrimp so when excreted; it becomes an expensive fertilizer that promotes the growth of pond populations of bacteria, and plankton (Moriarty, 1997). Ammonia (NH_3) is the main nitrogenous product excreted by crustaceans (Dall *et al.*, 1990). In intensive aquaculture systems, the toxicity of excreted N compounds becomes the limiting parameter once adequate dissolved oxygen levels are maintained (Colt & Armstrong, 1981). Unionized NH_3 becomes toxic since it has high lipid solubility and is able to diffuse across the cell membrane (Chen & Kou, 1993). Interactions between various N components are complex and difficult to integrate; modeling can improve our ability to evaluate this complexity. Modeling has been used as an approach to examine N dynamics in aquaculture systems by different authors (Lorenzen *et al.*, 1997; Paez-Osuna *et al.*, 1997; Montoya *et al.*, 1999a, 1999b). Steady state mass balance models provide information about the relation between N inputs and outputs, describing crop management overall efficiency and allowing comparisons. However they do not take into account the time-dynamic nature of N cycle in the commercially producing pond. Nitrification, volatilization, and re-mineralization were described as first-order rate processes (Lorenzen *et al.*, 1997). The objective of this study is to describe mathematically,

the fate of feed N inputs to the intensive white shrimp ZWE production ponds during crop. We adapted the Burford & Lorenzen (2004), model that describes the principal N transformation processes, and after calibration and validation in the ZWE white shrimp ponds, it was used to identify alternatives to improve yield.

MATERIALS AND METHODS

Three commercial ponds of three farms located in Coquimatlán (E11 and E21 with water sourced from a river and a well, respectively) and Tecomán (E2 with water sourced from a river, Zanja Prieta), Colima, were studied. Shrimp farms pond label was maintained in this work. E2 Pond is located 5.6 km from the sea, ponds E11 and E21 are within 48 km of the sea. Ponds were stocked in late March with the same postlarvae (PL) origin. In Table 1 are the production futures of the ponds as dimensions and load capacity. Water in all the three ponds was supplied to fill the ponds and to maintain the water level lost from seepage or evaporation, there was no precipitation during the experiment. Total cation concentration (TCC) of pond water was obtained with atomic absorption analysis in a VARIAN AA-220FS spectrophotometer (APHA, 1989). Salinity and TCC are directly proportional (Castillo-Soriano *et al.*, 2010). No water was discharged from the pond's spillways until shrimp harvest. These ponds had clay soils. During crop oxygen in water was above 3 mg L^{-1} at all times. Paddle wheel aeration was first applied to ponds when 35 kg $\text{ha}^{-1} \text{day}^{-1}$ of feed was demanded by the growing shrimp population. Then, one horse power (hp) was employed for every 7 kg of feed $\text{ha}^{-1} \text{day}^{-1}$. Pond E11 employed, towards the end of the culture cycle, aeration of 35 hp ha^{-1} while receiving 238 kg of feed $\text{ha}^{-1} \text{day}^{-1}$. Pond E21 employed aeration of 25 hp ha^{-1} while receiving 185 kg of feed $\text{ha}^{-1} \text{day}^{-1}$. Pond E2 employed aeration of 12 hp ha^{-1} while receiving 80 kg of feed $\text{ha}^{-1} \text{day}^{-1}$. Feed (sinking pellets) was applied twice daily evenly in all ponds. Ten feeding trays of 35 cm^2 per ha were used to monitor consumption. The ponds were fed with commercial feed (NASA) containing 35% of protein in ponds E11 and E21, and 25% of protein in pond E2 through the growing season. N content in shrimp and feed was obtained with the Kjeldhal method (Lynch & Barbano, 1999) from samples at the time of harvest for each pond. The relation between the applied N in dry feed, and the recovered N in shrimp was determined and used for comparisons to other reports in the discussion section. Production data were obtained from farm records. These data correspond to

pond surface area (Sup), pond deepness (z), stocking density (PL m^{-2}), shrimp biomass harvested (ton), fertilizer application, total amount of feed applied and finally total mortality. Daily mortality (M) was calculated from total mortality and crop duration. Feed conversion rate (FCR) is the total amount of dry feed delivered relative to shrimp biomass harvested. Shrimp biomass (B) density was calculated as the weight (g) of shrimp carried in a liter (L) of pond water. Shrimp growth was monitored every week with 100 shrimps captured using a castnet, and weighed to determine the average shrimp weight at each pond. To model the fate and dynamical behavior of N inputs in ponds, growing *L. vannamei*, practicing ZWE, the abundance in N containing chemical and biological structures were monitored periodically from stocking to harvest. In order to perform our task, pH and temperature were measured *in situ*, with a Horiba water checker U-10 potentiometer.

Additionally, three 0.5 L water samples were collected at morning in each pond, one meter from the spillway dike at 30 cm depth and transported in ice to the laboratory within three hours. Physical measurements and samples were matched for the dynamics analysis. To determine total Chlorophyll-*a* concentrations, 50 mL of each water sample was filtered with a milli-pore cellulose membrane of 0.45 μm . The membrane was introduced in a solution containing 9 mL acetone and 1 mL of distilled water. The solution was refrigerated for a week and analyzed in a Jenway 6500 spectrophotometer with a detection limit of 0.01 $mg L^{-1}$ (Strickland & Parsons, 1968). The inorganic phosphorous (P) and N ($NO_2^-+NO_3^-$, NH_4^+ and PO_4^{3-}) concentrations were evaluated, from the filtered water, using a Skalar ion auto-analyzer with a detection limit of 0.01 μm . Ammonia (NH_3) concentration was estimated by relating measured NH_4^+ , pH and temperature of the same sample event, using the aquatic equilibrium equation: $NH_3 + H_2O = NH_4^+ + OH^-$ (Trussell, 1972).

The N dynamical model (NDM): To describe the dynamical behavior of the N in the monitored ponds, a mathematical model of N-dynamics proposed by Burford & Lorenzen (2004) for tiger shrimp (*Penaeus monodon*) cultured intensively with WE, was adapted to *Litopenaeus vannamei* intensive culture practicing ZWE. The model requires the absence of herbivores (not planktonic), that all pond N inputs come exclusively from the feed and that shrimp and phytoplankton growth is not limited by the lack of oxygen or P. In pond E2, N was also applied as fertilizer (Table 1), therefore, this pond is not used for the model adaptation but serves the purpose of validating the white shrimp growth equation and food

conversion rates comparisons. The differences between the shrimp culture conditions reported by Burford & Lorenzen (2004), in Australia and the sampled ponds were: the shrimp species, the WE practices, the water temperature and salinity. The commercial farms in Colima and Australia shared management practices such as supplemented aeration and tray monitoring feeding.

Mathematical NDM in intensive ZWE shrimp ponds is given by a set of five coupled differential equations representing the main N components (Fig. 1).

$$\frac{dX_1}{dt} = qaN_0e^{-Mt}W_t^b + rX_4(n+v)X_1 - \frac{(gmaxLight \frac{X_1+X_2}{(X_1+X_2)+K_{S_N}})cX_3X_1}{X_1+X_2} \quad (1)$$

$$\frac{dX_2}{dt} = nX_1 - \frac{(gmaxLight \frac{X_1+X_2}{(X_1+X_2)+K_{S_N}})cX_3X_1}{X_1+X_2} \quad (2)$$

$$\frac{dX_3}{dt} = (gmaxLight \frac{X_1+X_2}{(X_1+X_2)+K_{S_N}})X_3 - sX_3 \quad (3)$$

$$\frac{dX_4}{dt} = scX_3 - rX_4 \quad (4)$$

$$\frac{dX_5}{dt} = (1-q)aN_0e^{-Mt}W_t^b \quad (5)$$

where $X_1 = NH_3$ concentration ($mg L^{-1}$), $t =$ time (day); $q =$ proportion of N waste entering the water as X_1 (With the remainder entering the water as X_5); $aN_0e^{-Mt}W_t^b$ is the total N waste input per unit time ($mg g^{-1} day^{-1}$); $r =$ remineralization rate of X_1 in the sludge (day^{-1}); $X_4 =$ mass of N (mg) in the sludge L^{-1} of pond water; $n =$ nitrification rate (day^{-1}); $v =$ volatilization rate (day^{-1}); $X_2 = NO_2^-+NO_3^-$ concentration ($mg L^{-1}$); $X_3 =$ Chlorophyll-*a* concentration ($mg L^{-1}$); concentrations were obtained at a fixed “ $c = N/X_3$ ratio of phytoplankton”; $s =$ sedimentation rate of phytoplankton (day^{-1}); $X_5 =$ dissolved organic N ($mg L^{-1}$). The total N waste input $aN_0e^{-Mt}W_t^b$ was assumed to be proportional to the metabolism of shrimp population (Burford & Lorenzen, 2004); where a is the total N waste (X_1 , and X_5) input rate ($mg g^{-1} day^{-1}$), and it was determined as the value for which N input over the cycle equaled the total feed N not incorporated to the shrimp tissue. The allometric scaling factor of metabolism is b . Shrimp population size in shrimp L^{-1} is given by the following exponential mortality model: N_0e^{-Mt} , where N_0 is the stocking density (PL L^{-1}) and M is the mortality rate (day^{-1}) of shrimp. Shrimp mean weight W_t is given by a von Bertalanffy growth function (Gulland, 1983):

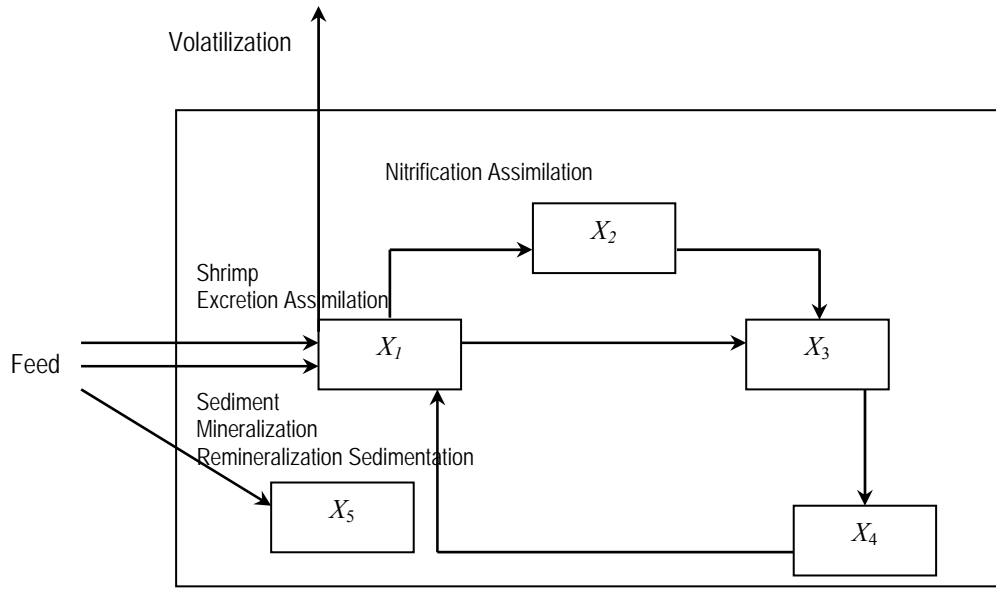


Figure 1. Represents the N transformations and removals in intensive shrimp (*Litopenaeus monodon*) ponds with ZWE NDM. Arrows represent pathways and boxes indicate the key N components represented as stated variables in the model: X_1 = total ammonia, X_2 = nitrites and nitrates, X_3 = Chlorophyll-*a*, as a measure of phytoplankton, and hence N in algae biomass, X_4 = dissolved N organic, and X_5 = N buried in sludge.

$$W_t = (W_\infty^{1/3} - (W_\infty^{1/3} - W_0^{1/3})e^{-Kt})^3 \quad (6)$$

where W_∞ (g) and K (day^{-1}) are the growth parameters of white shrimp maximum weight and metabolic coefficient. The $W_\infty = 55$ g was established by adding five grams from the largest shrimp of a population of 5,000 *Litopenaeus vannamei* eight-months old shrimps, grown at a density of 2 shrimp m^{-2} in low salinity at Coquimatlán, Colima. In the three studied ponds the shrimp average weight was used to determine metabolic growth coefficient K , through numerical fitting. W_0 (g) is the weight at stocking. Phytoplankton growth rate is defined as: $gmaxLight \frac{X_1+X_2}{(X_1+X_2)+K_{SN}}$, where $gmax$ is the maximum growth rate in the absence of any limitation. $Light$, is the light limitation coefficient given by the integral of Steel's (1962) light inhibition model over the water column, with light conditions defined by the Lambert-Beer law.

$$Light = \frac{e \left(\exp \left(\frac{I_0}{I_{sat}} \exp(-kX_3X_3 + k_{other}z) \right) \right) - \exp \left(\frac{I_0}{I_{sat}} \right)}{kX_3X_3 + k_{other}} \quad (7)$$

where I_0/I_{sat} is the ratio of the surface light intensity to the saturating light intensity, k is the extinction coefficient (m^{-1}) and z is the pond depth (m). The light extinction coefficient is the addition of extinction due to X_3 , and extinction coefficient due to other sources: $kX_3X_3 + k_{other}$, where kX_3 is the extinction per unit of X_3 concentration ($\text{m}^{-1} \text{mg}^{-1}$) and k_{other} is the extinction due

to other sources. N limitation is defined by the Michaelis-Menten model as: $\frac{(X_1+X_2)}{(X_1+X_2)+K_{SN}}$, where K_{SN} (mg L^{-1}), is the half saturation constant for N. We assumed that phytoplankton assimilates both X_1 and X_2 in proportion to their relative concentrations in the water column. The model was implemented with MATLAB® R2010b. After adapting the model two management strategies that increase $N_{(0)}$ were proposed for the sake of increasing the economic profitability in the ZWE farms and to hypothesize in the dynamics of N components in ponds using the NDM. We defined yield (Y) as the daily average biomass (B) (grams of shrimp L^{-1}) generated: $Y = B/t_f$, where $B = N_{t_f}W_{t_f}$ at harvest, t_f is the crop length in days, N_t is in shrimps L^{-1} and W_t is in grams. The water lost in ZWE ponds by seepage and evaporation is replaced with water from pond supply systems.

RESULTS

Mean total cation concentration in ponds E2, E11, E21 were 319, 562.5, 617 mg L^{-1} with standard deviations of 25, 24 and 31 mg L^{-1} respectively. In the three ponds calcium was the most abundant cation, followed by sodium, magnesium and potash. In Table 1, pond futures production data and N balance are presented. Adequate oxygen levels of over 3 mg L^{-1} and the

Table 1. General pond features production data and N balance.

Culture features in ponds	E2	E11	E21
Pond area (ha)	1.8	1.3	2.2
Pond depth (m)	1.2	1.4	1.5
Pond volume ($L \times 10^6$)	21.6	18.2	33
Total cation concentration at stocking ($mg L^{-1}$)	319	563	617
Salinity (ppt)	0.44	1.03	1.16
Average temperature ($^{\circ}C$)	31.3	28.4	28.4
Maximum aeration capacity installed ($hp ha^{-1}$)	12	35	30
Stocking density ($PL m^{-2}$)	31.2	60.2	58.5
Stocking density; N_0 ($PL L^{-1}$)	0.026	0.043	0.039
Total crop mortality (%)	60	28.7	41.2
Daily mortality (M)	0.0056	0.0023	0.0033
Pond shrimp production ($ton ha^{-1}$)	2.5	7.7	6.17
Growing time t_f (days)	108	125	125
Shrimp weight (W_i) at harvest (g)	19.6	17	15.6
Shrimp biomass ($g L^{-1}$)	0.208	0.55	0.411
Shrimp harvested (kg)	4500	10010	13574
Nitrogen in shrimp harvested (kg)	126	280	380
Nitrogen in applied feed (kg)	216	660	896
Nitrogen applied in fertilizer (kg)	462	0	0
Feed/shrimp waste nitrogen ratio (a)	1.7	2.3	2.3
Feed conversion ratio (FCR)	1.2	1.2	1.2

absence of not planktonic herbivores requirements are fulfilled by each of the three ponds. The relation N/P was always less than 10 in all ponds (Table 2) assuring that P is not limiting phytoplankton growth. A decreasing N/P ratio was observed as crop evolved in the ponds (Table 2). N content in feed used in ponds E11 and E21 was of 5.53% and of 4.01% in pond E2. The shrimp N content was 2.8% so we know how much N was retained by the crop. Table 1 shows N input in feed and the assimilated N by the shrimp biomass. Figure 2 shows the proper fitting of the function W_i to the shrimp average weight during crop. K values were obtained from equation 6 through numerical fitting. Table 3 shows parameter values used in the NDM for ponds E11 and E21 only. White shrimp population parameters obtained, were used in the NDM, and numerical fitting was performed to describe the fate of N inputs. The value of $gmax$ was selected to best fit the X_3 experimental data of ponds E11 and E21. Figure 3, shows the numerical simulation corresponding to the NDM as well as experimental data sampled during crop. It reveals a good agreement between experimental data and the NDM, implying the correct selection of the parameter

values. Figure 4 shows the evolution of the pond carrying B , and Figure 5 shows the dynamical behavior of X_1 , X_2 and X_3 of real and hypothetical production management strategies. Finally the model application for commercial production processes is presented in Table 4 where the comparison of different management strategies such as stocking density and best time to harvest can be established by comparing Y .

DISCUSSION

Feed demand and consequently N input, increased as shrimp biomass grew. Paddle wheel aerators prevented oxygen from falling under $3 mg L^{-1}$ and established a well mixed aquatic environment. Phytoplankton, zooplankton, nitrifying and heterotrophic bacteria share available N excreted from shrimp in ponds (Burford *et al.*, 2003). In turn, the accumulation of ammonia (X_1) first causes cessation of feeding of shrimp, and then subsequent population mortality (Chen *et al.*, 1990). In the case of white shrimp, PL in ecdysis stage dies if concentrations of X_1 exceed $10 mg L^{-1}$ (Frias-Espericueta *et al.*, 2000),

Table 2. Obtained parameters in studied ponds along growing season.

Pond	Time (day)	X_2	NH_4^+	NH_4^+	PO_4^{3-}	PO_4^{3-}	pH	T (°C)	NH_4^+	X_1	N/P	X_3	X_3
		(mg L^{-1})	(mg L^{-1})	SD	(mg L^{-1})	SD			NH_3	(mg L^{-1})		(mg L^{-1})	SD
E21	0	0	0.57	0.06	0.24	0.03	8.4	25	8.3	0.068	6.7	0.099	0.07
	5	0	0.549	0.07	0.23	0.01	7.6	25	45.5	0.012	5.9	0.0284	0.05
	28	0	0.313	0.05	0.14	0.02	9.6	29	1.3	0.241	10.0	0.043	0.09
	43	0	0.233	0.04	0.21	0.03	9.7	29	1.3	0.177	4.9	0.0738	0.08
	59	0	0.235	0.03	0.23	0.02	10	28	1.3	0.188	4.7	0.0929	0.06
	83	0	0.356	0.02	0.21	0.02	8.8	29	3.3	0.109	5.6	0.0978	0.14
	95	0	0.269	0.09	0.23	0.03	8.6	29	4.6	0.059	3.5	0.2323	0.1
	104	0	0.24	0.05	0.20	0.04	8.1	30	8.8	0.027	3.2	0.2019	0.2
	119	0	0.303	0.03	0.31	0.01	9	29	2.4	0.127	3.5	0.2816	0.18
	125	0	0.3	0.02	0.27	0.03	8.1	31	11.5	0.026	2.9	0.2323	0.18
E11	0	0	0.59	0.04	0.24	0.02	8.4	24	8.6	0.069	6.8	0.0408	0.09
	23	0	0.236	0.04	0.26	0.01	9.6	29	1.3	0.177	4.0	0.0095	0.06
	38	0	0.175	0.02	0.25	0.01	9.3	29	1.9	0.095	2.7	0.0626	0.08
	53	0	0.283	0.02	0.24	0.03	8.6	28	3.3	0.087	3.9	0.0672	0.1
	77	0	0.24	0.03	0.25	0.05	8.4	29	4.3	0.055	2.9	0.1686	0.09
	89	0	0.24	0.02	0.15	0.02	9	29	2.3	0.103	5.6	0.0352	0.07
	98	0	0.214	0.05	0.16	0.03	8.5	29	5.9	0.036	3.9	0.271	0.12
	113	0	0.143	0.01	0.19	0.02	8.9	30	2.3	0.063	2.7	0.2292	0.1
E2	0	0	0.342	0.02	0.18	0.02	8.9	30	2.3	0.15	6.9	0.1362	0.08
	29	0	0.259	0.02	0.22	0.01	10	31	1.1	0.233	5.6	0.0716	0.08
	56	0	0.259	0.04	1.54	0.02	9.8	33	1.2	0.222	0.8	0.0909	0.09
	71	0	0.182	0.02	2.89	0.04	9	31	2.2	0.082	0.2	0.0737	0.08
	82	0	0.196	0.03	0.15	0.02	9.5	31	1.4	0.137	5.7	0.0725	0.11
	95	0	0.195	0.02	0.24	0.03	8.7	32	2.6	0.074	2.8	0.0043	0.03

Decamp *et al.* (2003) found that water salinity did not seem to impact N dynamics significantly within the white shrimp culture environment, either directly (through the activity of nitrifying bacteria) or indirectly (through the N retention or excretion by shrimp).

NDM validation in the ZWE low salinity white shrimp ponds: In a pond that is receiving increasing amounts of feed, the phytoplankton density eventually limits its own growth by self shade (Eq. 7). The NDM predicts an accumulation of X_1 after X_3 is unable to keep growing. N is partially lost from the pond by volatilization of X_1 particularly in heavily aerated and high pH periods. A high pH range is 8 to 10 wherein the ammonia dissociates into ammonium ion when the pH drops as high as this is the case is maintained in the form toxic but is highly volatile (Boyd, 1990) and is referenced as v (Table 3). In the studied ponds, the

X_1 concentration did not rise during the production period, even when daily feeding was intensive. The X_1 increase was expected after harvest using the NDM (Fig. 3), implying that harvest was done in the appropriate moment, avoiding the critical period in which X_1 becomes toxic. In addition, the NDM showed that it could have been possible to leave the shrimp to grow longer but also that ponds could have been stocked at a higher density. Sub section 4.3 tackles such management options based on the NDM. Phytoplankton (X_3) growth plays a key role in shrimp aquaculture, driven gas exchange, pH and as the base of the food chain in the pond (Alonso-Rodriguez *et al.*, 2004).

Phosphate usually limits phytoplankton productivity in natural fresh water ecosystems (Baird, 1999) in general and in aquaculture ponds, soil absorbs P (Boyd & Munsiri, 1996) making it less available.

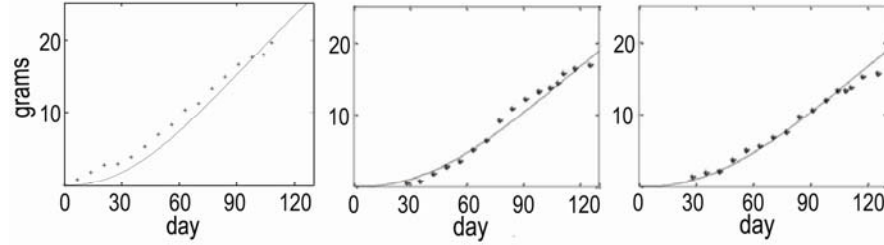


Figure 2. Graphics show, from left to right, the modeled shrimp average weight in the three Colima ponds. Dots stand for the experimental data whereas the continuous lines represent W_t from the growth function, with its respective values of k obtained by a fitting procedure.

Table 3. Parameter values in ponds E11 and E21.

Parameter description	Symbol	Units	E11	E21	Source
White shrimp population					
Shrimp growth coefficient	K	day ⁻¹	0.0089	0.0086	1
Shrimp maximum weight	W_{∞}	g	55	55	1
Shrimp stocking weight	W_0	G	0.005	0.005	1
Shrimp mortality	M	day ⁻¹	0.0023	0.0033	1
Stoking density	N_0	shrimp L ⁻¹	0.043	0.04	1
Nitrogen dynamics					
Waste N Input	A	mg g ⁻¹ day ⁻¹	2.3	2.3	1
Proportion of N entering as X_1	Q		0.9	0.9	2
Allometric scaling of X_1 excretion	B		0.75	0.75	3
Pond depth	Z	m	1.4	1.5	1
N half-saturation	K_{SN}	mg l ⁻¹	0.008	0.008	2
Sedimentation rate	s	day ⁻¹	0.8	0.8	2
Nitrification rate	n	day ⁻¹	0.15	0.15	2
Volatilization rate	v	day ⁻¹	0.05	0.05	2
Sludge remineralization rate	r	day ⁻¹	0.06	0.06	2
Phytoplankton parameters					
Maximum phytoplankton growth rate	g_{max}	day ⁻¹	1.9	1.9	4
Ratio surface/saturating light intensity	I_0/I_{sat}		2.4	2.4	2
N/ X_3 ratio of algae	c		13	13	2
Extinction coefficient Non- X_3	K_{other}	m ⁻¹	2.5	2.5	5
Extinction coefficient X_3	k_{X3}	m ⁻¹ mg ⁻¹	14	14	5

Sources: 1. Experimentally obtained, 2: Burford & Lorenzen (2004), 3: Burford & Williams (2001), 4: Numerical Fitting, 5: Burford (1997).

Redfield (1958) showed that if the relation $N/P < 16$, the lower P does not limit phytoplankton growth. In the studied ponds, N/P molar ratio was always less than ten (Table 2) so X_3 was not limited by low P abundance. Indeed, a decreasing N/P molar ratio is observed as crop evolves in the three ponds. It is known that the taxonomic composition of phytoplankton changes if nutrient proportions change (Burford, 1997) as with changing conditions of oxygen (Chapelle *et al.*, 2000). In particular, low salinity environments with a low N/P ratio carry a higher proportion of

smaller size phytoplankton, such as cyanophytes (Margalef, 1983).

In the model, since P is not limiting excreted N (X_1) is stored in the living pond algae (X_3) that increases in concentration until it's self-shaded. Concentrations of nitrites and nitrates (X_2) were nil in ponds during the entire crop, which implies that the removal rate of X_2 by X_3 , is higher than its production. Low values of X_2 sometimes bellow the limit of detection of the analytical procedure was found in several intensive farms in Sonora (Ruiz-Fernandes &

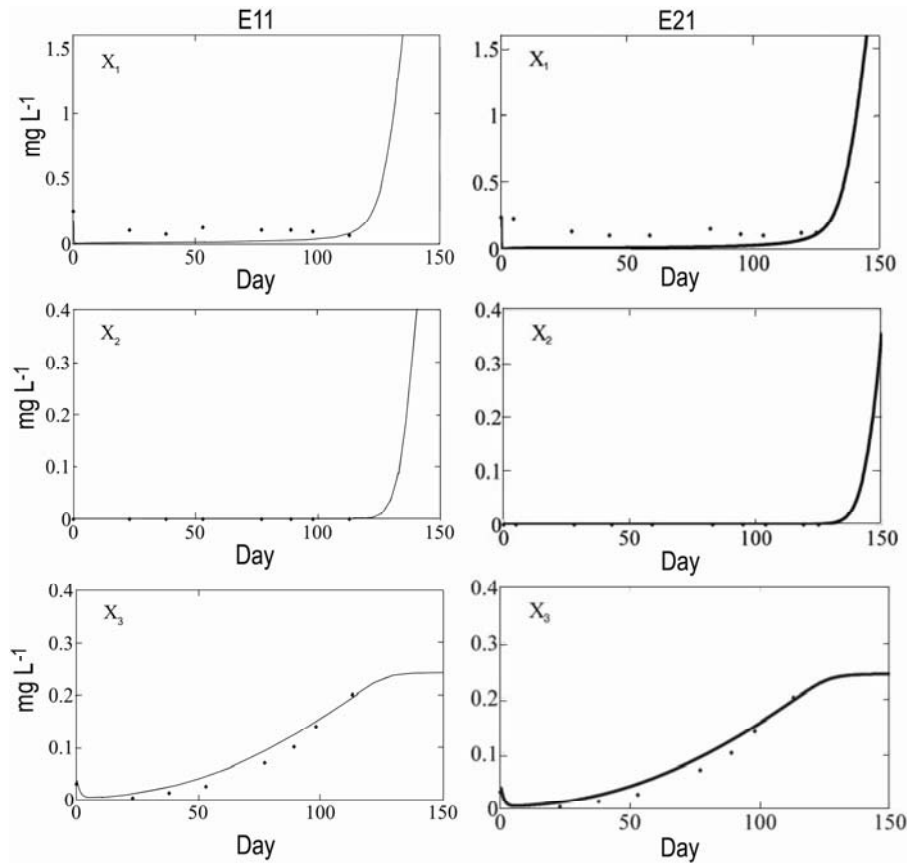


Figure 3. Experimental data and model of X_1 , X_2 , and X_3 from two ponds that fulfill the criteria required for the usage of NDM. Dots stand for the experimental data whereas continuous lines represent the dynamical model.

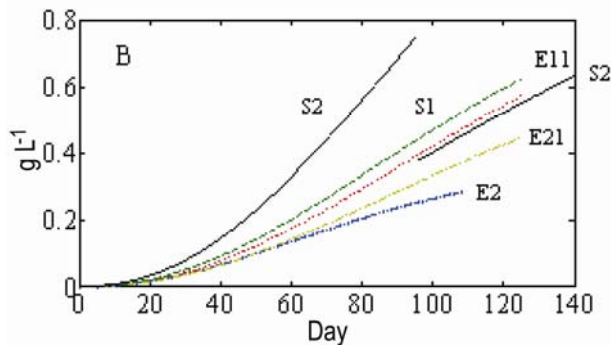


Figure 4. The plot shows the time evolution of the ponds carrying the shrimp biomass (B) as well as the proposed strategies. The continuous grey line stands for pond E2, the dash-dotted line corresponds to pond E21, dotted line stands for pond E11. The dashed line corresponds to strategy 1 (S1) and the continuous black line stands for strategy 2 (S2), doing a partial harvest of biomass and leaving the pond producing for a longer time.

Paez-Osuna, 2004). While nitrite-oxidizing bacteria are slow growing organisms (Ehrich *et al.*, 1995), a value of g_{max} of 1.9 was required to fit data of X_3 dynamic growth in ponds E11 and E21 (Table 3), as

compared to that employed in Australia of 1.4 (Burford & Lorenzen, 2004). This increment was in range of highly intensive farms with WE (Lorenzen *et al.*, 1997). Snares *et al.* (1986) found that the X_3 values were highest in ponds with continuous aeration as aeration circulation contributes to accelerate algal growth compared with undisturbed conditions.

Phytoplankton growth increases with, alga decreasing cell size (Kagami & Urabe, 2001) and temperature (Falkowsky & Raven, 1997; Burford, 1997). A high stirring capacity, low N/P ratio, and a greater temperature likely generated high small size cyanophyte dominated cell density and a higher g_{max} .

White shrimp daily growth coefficient K in all ponds was higher as compared to the tiger shrimp K , grown at 25°C (Burford & Lorenzen, 2004), since shrimp growth rate also increases with temperature (Yu & Bienfang, 2006).

Feed conversion rate FCR and comparative N balances: Ponds in Colima achieved a FCR of 1.2, working under ZWE. These systems were more efficient in the assimilation of the applied N in feed than the ponds using daily WE. In Australia (Burford

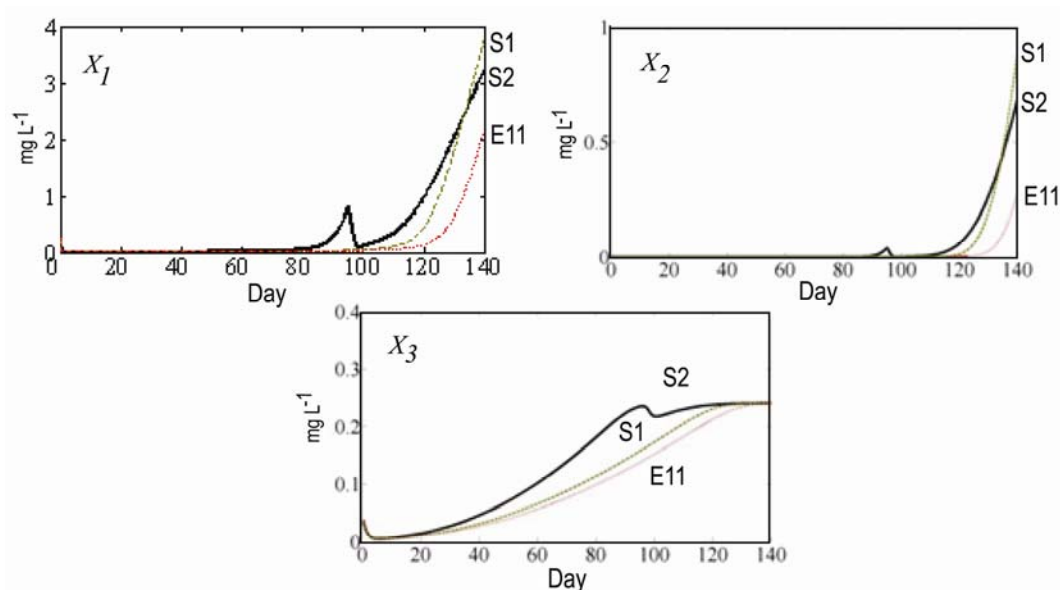


Figure 5. Plots show the dynamical behavior of X_1 , X_2 and X_3 , according to the NDM. The dotted line stands for pond E11, while the dashed line represents strategy 1 (S1) and the black line strategy 2 (S2).

Table 4. Comparison of the yield values of white shrimp populations, in studied ponds and proposed strategies of stocking and harvest, using the NDM to avoid N toxicity.

Pond	N_0	K	M	t_f	W_t at harvest	$Y(\text{g L}^{-1}\text{day}^{-1})$
E2	0.026	0.011	0.0056	108	19.6	2.6×10^{-3}
E21	0.04	0.0086	0.0033	125	15.6	3.5×10^{-3}
E11	0.043	0.0089	0.0023	125	17	4.6×10^{-3}
Strategy 1	0.053	0.0089	0.0033	125	17	5.0×10^{-3}
Strategy 2	0.08	0.0089	0.0033	140	11,18	7.2×10^{-3}

& Lorenzen, 2004) and Thailand (Brigs & Funge-Smith, 1994) a FCR of 1.9 was obtained growing tiger shrimp. In white shrimp production ponds using feeding trays and practicing WE FCR's were: 2.3, 2.7 in Texas (2 farms) (Samochoa *et al.*, 2004), 1.6 in Sinaloa (23 farms) (Lyle-Fritch *et al.*, 2006), and 1.78 in Sonora (1 farm) (Casillas-Hernandez *et al.*, 2006, 2007). Lower FCRs might be more related to the retention of nutrients in the pond system by not exchanging, than to the shrimp species. A higher FCR implies that less protein-derived N is retained from the shrimp population biomass. Whereas feed N recovery using WE in semi-intensive ponds was of 35% (Paez-Osuna *et al.*, 1997), 27.2% (Casillas-Hernandez *et al.*, 2006) in intensive ponds was 22% (Brigs & Funge-Smith, 1994). In contrast, this study yielded a feed N recovery of 43.4% in unfertilized ponds E11 and E21. Such results are consistent with the fact that shrimp fed with differing protein content had equal growth in

the presence of phytoplankton (Martínez-Córdoba *et al.*, 2003), yet in clear aquariums, shrimp grew faster with higher protein content (Leber & Pruder, 1988), implying the usage of nutrients from the environment and that the microalgae are consumed by shrimp (Gómez-Aguirre & Martínez-Córdoba, 1998), particularly as juveniles (Mishra *et al.*, 2007). In general, production is higher in what is considered poor water quality (high nutrient concentrations, high and unstable phytoplankton numbers, and high bacterial numbers) (Burford *et al.*, 2003). Forty to sixty percent of shrimp tissue comes from pond natural nutrients (Brown *et al.*, 1998; Focken *et al.*, 1998). Better yield and FCR is obtained in WE farms during rainy season because temperature and X_3 concentration are higher (Guerrero-Galvan *et al.*, 1999). We suggest that the lower FCR in ponds in Colima is a result of the excreted N remaining in the ZWE ponds food chain, allowing its later usage by the shrimps, instead of being flushed away with WE.

Management strategies: Better management strategies in ZWE ponds aim to increase Y , growing shrimp in such a manner that X_I is always in a “safe” level, that for *L. vannamei* can be set at 5 mg L⁻¹ (Frias-Espicueta *et al.*, 1999). In the three ponds analyzed the shrimp was harvested before X_I achieved toxic levels. The NDM allows the analysis of higher stocking densities that can safely increase Y . Y increases if $N_{(0)}$ or K increase, and if M decreases. We modeled two alternative stocking strategies and compared their outcome to one another. In this analysis K (depends on temperature) and M needed to be set arbitrarily or based on previous pond records.

In Strategy 1 a $N_{(0)}$ of 0.053 PL L⁻¹ is used, (increased 32.5% as compared to E11), all of the shrimp biomass is harvested at the end of the crop, at day 125 ($t_{(125)}$) (Table 4). M was set at 0.0033 (arbitrarily), K and t_f remain the same as the strategy used in commercial pond E11.

In Strategy 2, $N_{(0)}$ of 0.08 PL L⁻¹, is used, (increased 100% as compared to E11), 40% of the shrimp biomass is harvested at day 95 ($t_{(95)}$), when the shrimp had achieved a weight of 11 g (selected, because the size is adequate for the national market). The remainder of the shrimps are left 140 days ($t_{(140)}$). K and M are the same as strategy 1. In figure 4, it is possible to appreciate the shrimp biomass (B) dynamics of studied ponds as compared with the two proposed strategies that increased N_0 . In figure 5, one can see the effect of such management practices as compared to E11, in a ZWE white shrimp pond N dynamic. Ponds can produce higher Y without risking N toxicity. We noticed a decrease in X_I , X_2 and X_3 in strategy 2, when a portion of the shrimp biomass is removed and consequently, N input in feed and waste N is reduced after first harvest. In order to have a better comparison between the strategies commonly used in Colima and those proposed in this paper we estimate the Y values in each case. The comparison is presented in Table 4. We see that the best Y is provided by strategy 2, in which the stocking density is increased 100% and a partial harvest is conducted prior to final harvest.

CONCLUSIONS

We found that the P concentrations do not limit the phytoplankton growth during crop in the ZWE ponds. Ammonia N transformation is done rapidly by bacteria and phytoplankton, preventing X_I from accumulating and becoming toxic at shrimp biomass of 0.8 g L⁻¹. Applied N is mostly stored in pond populations particularly of shrimp and phytoplankton. When producing at low salinity and applying feed and

aeration, phytoplankton maximum growth rate g_{max} is high. The NDM predicts that is plausible to increase shrimp Y , without risking ammonia toxicity, by increasing stocking density and harvesting shrimp partially before a final harvest. Because of lower FCRs, high standing crops have been achieved in ZWE ponds. It is also pertinent to mention the no P limitation in crops as this is beyond the scope of work and observed indirectly.

ACKNOWLEDGMENTS

This work is dedicated to the memory of our outstanding collaborator Professor A.O. Meyer-Willerer, who passed away in August 2008. F.A. Castillo-Soriano thanks CONACYT for the grant that partially supported him during the research period. V. Ibarra-Junquera thanks PROMEP for the financial support. The authors are thankful to Professor H.C. Rosu for his technical comments.

REFERENCES

- Alonso-Rodriguez, R., F. Paez-Ozuna & I. Garate-Lizarraga. 2004. El fitoplancton en la camaronicultura: importancia de un buen manejo. Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México y Comité Estatal de Sanidad Acuicola de Sinaloa, Sinaloa, 147 pp.
- American Public Health Association (APHA). 1989. Standard method for the examination of water and waste water. American Public Health Association. Washington D.C.
- Baird, C. 1999. Environmental chemistry. W.H. Freeman and Company, New York, 476 pp.
- Boyd C.E. 1999. Aquaculture sustainability and environmental issues. World Aquacult., 30(2): 10-13.
- Boyd, C.E. 1990. Water quality in ponds for aquaculture, Alabama agricultural experiment station, Auburn University, Auburn, 482 pp.
- Boyd, C.E. & P. Munsiri. 1996. Phosphorus adsorption capacity and availability of added phosphorus in soils from aquaculture areas in Thailand. J. World Aquacult. Soc., 27: 160-167.
- Brigs, M.R.P. & S.J. Funge-Smith. 1994. A nutrient budget of some intensive marine shrimp ponds in Thailand. Aquacult. Fish. Manage., 25: 789-811.
- Brown, R.M., J.K. Jeffrey & G.A. Dunstan. 1998. Nutritional properties of microalgae for mariculture. Aquaculture, 151(1-4): 315-331.
- Burford, M. 1997. Phytoplankton dynamics in shrimp ponds. Aquacult. Res., 28: 351-360.

- Burford, M.A. & K. Lorenzen. 2004. Modeling nitrogen dynamics in intensive shrimp ponds: the role of sediment remineralization. *Aquaculture*, 229: 129-145.
- Burford, M.A. & K.C. Williams. 2001. The fate of nitrogenous waste from shrimp feeding. *Aquaculture*, 198: 79-93.
- Burford, A.M., P.T. Thompson, R.P. McIntosh, R.H. Bauman & D.C. Pearson. 2003. Nutrient and microbial dynamics in high intensity, zero-exchange shrimp ponds in Belize. *Aquaculture*, 219: 393-411.
- Casillas-Hernandez, R., R.F. Magallon-Barajas, G. Portillo-Clarck & F. Paez-Osuna. 2006. Nutrient mass balances in semi-intensive shrimp ponds from Sonora, México using two feeding strategies: trays and mechanical dispersal. *Aquaculture*, 258: 289-298.
- Casillas-Hernandez, R., T. Nolasco-Soria, O. Garcia-Galano, F. Carrillo-Farnes & F. Paez-Osuna. 2007. Water quality chemical fluxes and production in semi-intensive pacific white shrimp (*Litopenaeus vannamei*) culture ponds utilizing two different feeding strategies. *Aquacult. Eng.*, 36: 105-114.
- Castillo-Soriano, F.A., V. Ibarra-Junquera, A. Olivos-Ortiz, F.J. Barragan-Vasquez & A.O. Meyer-Willerer. 2010. Influence of water supply chemistry on white shrimp (*Litopenaeus vannamei*) culture in low-salinity and zero water exchange ponds. *Pan Am. J. Aquatic. Sci.*, 5(3): 376-386.
- Chapelle, A., A. Ménesguen, J.M. Deslous-Paoli, P. Souchu, N. Mazouni, A. Vaquer & B. Millet. 2000. Modelling nitrogen, primary production and oxygen in a Mediterranean lagoon. Impact of oysters farming and inputs from the watershed. *Ecol. Model.*, 127: 161-181.
- Chen, J.C. & Y.Z. Kou. 1993. Accumulation of ammonia in the haemolymph of *Penaeus monodon* exposed to ambient ammonia. *Aquaculture*, 109: 177-185.
- Chen, J.C., P.C. Liu & S.C. Lei. 1990. Toxicity of ammonia and nitrite to *Penaeus monodon* adolescents. *Aquaculture*, 89: 127-137.
- Colt, J.E. & D.A. Armstrong. 1981. Nitrogen toxicity to crustaceans, fish and mollusks. Proceedings of the Bio-engineering Symposium for Fish Culture. Fish culture section of the Transactions of the American Fisheries Society, (FCS Publ. 1): 34-47.
- Dall, W., B.J. Hill, P.C. Rotlishberg & D.J. Sharpless. 1990. The biology of the Penaeidae. In: J.H.S. Blaxter & A.J. Southward (eds.). *Adv. Mar. Biol.*, 27: 1-489.
- Decamp, O., J. Cody, L. Conquest, G. Delanoy & A.G.J. Tacon. 2003. Effect of salinity on natural community and production of *Litopenaeus vannamei* (Boone), within experimental zero-water exchange culture systems. *Aquacult. Res.*, 34: 345-355.
- Ehrlich, S., D. Beherens, E. Lebedeva, W. Ludwin & E. Bock. 1995. A new obligate chemolithoautotrophic, nitrite-oxidizing bacterium, *Nitrospira moscoviensis* sp. nov. and its phylogenetic relationship. *Archiv. Microbiol.*, 164: 16-23.
- Falkowski, P.G. & J.A. Raven. 1997. Aquatic photosynthesis. Blackwell Science, Malden, 375 pp.
- Focken, U., A. Groth, R.M. Coloso & K. Beker. 1998. Contribution of natural food and supplemental feed to the gut content of *Penaeus monodon* Fabricius in a semi-intensive pond system in the Philippines. *Aquaculture*, 164(1-4): 105-116.
- Frias-Espericueta, M.G., M. Harfush-Melendez, I. Osuna-Lopez & F. Paez-Osuna. 1999. Acute toxicity of ammonia to juvenile shrimp *Penaeus vannamei* Boone. *Bull. Environ. Contam. Toxicol.*, 62: 646-652.
- Frias-Espericueta, M.G., M. Harfush-Melendez & F. Paez-Osuna. 2000. Effects of ammonia on mortality and feeding of post larvae shrimp *Litopenaeus vannamei*. *Bull. Environ. Contamin. Toxicol.*, 65: 98-113.
- Guerrero-Galván, S.R., F. Paez-Osuna, A.C. Ruiz-Fernandez & R. Espinoza-Angulo. 1999. Seasonal variation in the water quality and chlorophyll-*a* of semi-intensive shrimp ponds in a subtropical environment. *Hydrobiologia*, 391: 33-45.
- Gómez-Aguirre, S. & L.R. Martínez-Córdova. 1998. El fitoplancton. In: L.R. Martínez-Córdova (ed.). *Ecología de los sistemas acuícolas*. AGT Editio. S.A., Mexico D.F., pp. 77-94.
- Gulland, J.A. 1983. Fish stock assessment: a manual of basic methods. Wiley, Chichester, 223 pp.
- Hopkins, J.S., R.D. Hamilton II, P.A. Sandfier, C.L. Browdy & A.D. Stokes. 1993. Effect of water exchange rate on production, water quality, effluent characteristic and nitrogen budget of intensive shrimp ponds. *J. World Aquacult. Soc.*, 24: 304-320.
- Kagami, M. & J. Urabe. 2001. Phytoplankton growth rate as function of cell size: an experimental test in Lake Biwa. *Limnology*, 2: 111-117.
- Kautsky, N., P. Ronnback, M. Tedengren & M. Troell. 2000. Ecosystem perspective on management of disease in shrimp pond farming. *Aquaculture*, 191: 145-161.
- Leber, K.M. & G.D. Pruder. 1988. Using experimental microcosms in shrimp research: The growth-enhancing effect of shrimp pond water. *J. World Aquacult. Soc.*, 19(4): 247.

- Liebig, J. 1840. Chemistry and its applications to agriculture and physiology. Taylor and Walton, London, 407 pp.
- Lorenzen, K., J. Struve & J. Cowan. 1997. Impact of farming intensity and water management on nitrogen dynamics in intensive pond culture: a mathematical model applied to Thai commercial shrimp farms. *Aquacult. Res.*, 28: 493-507.
- Lovel, T. 1988. Nutrition and feeding of fish. Van Nostrand Reinhold, New York, 210 pp.
- Lyle-Fritch, L.P., E. Romero-Beltran & F. Paez-Osuna. 2006. A survey on use of the chemical and biological products for shrimp farming in Sinaloa (NW Mexico). *Aquacult. Eng.*, 35: 135-146.
- Lynch, J.M. & D.M. Barbano. 1999. Kjeldahl nitrogen analysis as a reference method for protein determination in dairy products. *J. AOAC International*, 82(6): 1389-1398.
- Margalef, R. 1983. *Limnología*. Omega, Barcelona, 1010 pp.
- Martínez-Córdoba, L.R., A.C. Campaña-Torres & M.A. Porchas-Cornejo. 2003. Dietary protein level and natural food management in the culture of blue (*Litopenaeus stylirostris*) and white (*Litopenaeus vannamei*) shrimp in microcosms. *Aquacult. Nutr.*, 9: 155-160.
- Montoya, R.A., A.L. Lawrence, W.E. Grant & M. Velasco. 1999a. Simulation of nitrogen dynamics and shrimp growth in an intensive shrimp culture system: effects of feed and feeding parameters. *Ecol. Model.*, 122: 81-95.
- Montoya, R.A., A.L. Lawrence, W.E. Grant & M. Velasco. 1999b. Simulation of inorganic nitrogen dynamics and shrimp survival in an intensive shrimp culture system. *Aquacult. Res.*, 33: 81-94.
- Moriarty, D.J.W. 1997. The role of microorganisms in aquaculture ponds. *Aquaculture*, 151: 333-349.
- Mishra, J.K., T.M. Samocha, S. Patnaik, M. Speed, R.L. Gandy & A. Ali. 2007. Performance of intensive nursery system for the Pacific white shrimp, under limited discharge condition. *Aquacult. Eng.*, 38: 2-15.
- Naylor, R.L., R.J. Goldburg, H. Mooney, M. Bevrige, J. Clay, C. Folke, N. Kautsky, J. Lubachenco, J. Primavera & M. Williams. 1998. Nature subsidies to shrimp and salmon farming. *Science*, 282: 883-884.
- Naylor, R.L., R.J. Goldburg, J.H. Primavera, N. Kautsky, M.C.M. Bevrige, J. Clay, C. Folke, J. Lubachenco, H. Mooney & M. Troell. 2000. Effect of aquaculture on world fish supplies. *Nature*, 405: 1017-1024.
- Paez-Osuna, F. 2001a. The environmental impact of shrimp aquaculture: causes, effects and mitigating alternatives. *Environ. Manage.*, 28: 131-140.
- Paez-Osuna, F. 2001b. The environmental impact of shrimp aquaculture: a global perspective. *Environ. Pollut.*, 112: 229-231.
- Paez-Osuna, F., S.R. Guerrero-Galvan, A.C. Ruiz-Fernandez & R. Espinoza-Angulo. 1997. Fluxes and mass balances of nutrients in semi-intensive shrimp farm in north-western Mexico. *Mar. Pollut. Bull.*, 34: 290-297.
- Paez-Osuna, F., A. Garcia, F. Flores-Verdugo, L.P. Lyle-Fritch, R. Alonso-Rodríguez & A.C. Ruiz-Fernández. 2003. Shrimp aquaculture development and the environment in the Gulf of California ecoregion. *Mar. Pollut. Bull.*, 46: 806-815.
- Redfield, A.C. 1958. The biological control of chemical factors in the environment. *Am. Sci.*, 46: 205-222.
- Ruiz-Fernandez, A.C. & F. Paez-Osuna. 2004. Comparative survey of the influent and effluent water quality of shrimp ponds on Mexican farms. *Wat. Environ. Res.*, 76: 5-14.
- Samocha, T.M., I.M. Lopez, E.R. Jones, S. Jackson & A.L. Lawrence. 2004. Characterization of intake and effluent waters from shrimp intensive and semi-intensive shrimp farms in Texas. *Aquacult. Res.*, 35: 321-339.
- Snares, R.C., S.A. Katase, A.W. Fast & K.E. Carpenter. 1986. Water quality dynamics in brackishwater shrimp ponds with artificial aeration and circulation. The first Asian fisheries forum. In: J.L. Maclean, L. Dizon, B. Hosillos & L. Vphilippines (eds.). *Asian Fisheries Society, Manila, Philippines*, pp. 83-86.
- Strickland, J.D.H. & T.R. Parsons. 1968. A practical handbook of seawater analysis. *Bull. Fish. Res. Bd. Can.*, Ottawa, 167: 293 pp.
- Thoman, E.S., E.D. Ingall, D.A. Davis & C.R. Arnold. 2001. A nitrogen budget for a closed, recirculating mariculture system. *Aquacult. Eng.*, 24: 195-211.
- Trussell, R.P. 1972. The percent un-ionized ammonia in aqueous ammonia solutions at different pH levels and temperatures. *J. Fish. Res. Bd. Canada*, 29: 505-507.
- Yu, R. & P. Bienfang. 2006. Predicting shrimp growth: artificial neural network *versus* nonlinear regression models. *Aquacult. Eng.*, 34: 6-30.