An evaluation of intraspecific competition for Pacific white shrimp Penaeus vannamei *(Boone) in extensive/semi-intensive ponds*

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An evaluation of intraspecific competition for Pacific white shrimp *Penaeus vannamei* (Boone) in extensive/semi-intensive ponds

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Abstract The hypothesis that intraspecific competition affects survival and growth during the culture and harvest at extensive/semi-intensive *Penaeus vannamei* shrimp ponds was evaluated. Thus, the effect of stocking density on the biomass, shrimp average weight, survival, and economic performance during the culture (133 days) and at the harvest of the *P. vannamei* shrimp was investigated in 400 m^2 earthen ponds. In order to reduce the likelihood of infectious diseases, shrimp received preventive health treatments (probiotics and β-1,3/1,6-glucans) during all culture phases. In this way, the effect of density on the intraspecific competition for space/food was isolated. Ponds stocked at 6, 9, and 12 shrimp m^{-2} showed competition-dependent growth. Ponds stocked at 12 shrimp m^{-2} presented a mortality (12 %) between days 76 and 99. Competition, and accordingly individual growth reduction, could have begun at day 76 at a density of 5 shrimp m^{-2}. Survival was significantly higher at 6 shrimp m^{-2} (84.2 ± 6.2 %) compared with the 12 shrimp m^{-2} (64.8 ± 12.4 %) treatment, while no significant differences in yield were observed between both treatments. Ponds stocked at 3 and 6 shrimp m^{-2} presented the best benefit–cost rates. The optimal shrimp density during the experimental culture was 5 shrimp m^{-2}. Given the experimental conditions and considering the fraction of density-independent mortality observed, the optimum stocking density was found to be 6 shrimp m^{-2}.

Keywords B–N graph · Extensive/semi-intensive ponds · Intraspecific competition · Optimal stocking density · Pacific white shrimp *Penaeus vannamei* (Boone)

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Abbreviations

ANOVA	Analysis of variance
B	Biomass
B–N graph	Biomass–density graph
CENAIM	Centro Nacional de Acuicultura e Investigaciones Marinas
ESPOL	Escuela Superior Politécnica del Litoral
FCR	Feed conversion rate
N	Density
N5	Nauplii5
OSD	Optimal stocking density
PL	Postlarvae
SD	Standard deviation
WSD	White spot disease
WSSV	White spot syndrome virus

Introduction

After the collapse of the cultured shrimp industry (early 2000 s), provoked by the white spot disease (WSD), Ecuadorian production has significantly improved. Among other reasons, the recovery has been attributed to a reduction in stocking densities down to extensive levels, producing larger shrimp at harvest and greater profitability (Stern and Sonnenholzner 2011). Despite the improvement of Ecuadorian shrimp production, WSD outbreaks still represent the main infectious disease problem at the pond level.

Ecuadorian shrimp farmers currently apply immunostimulants and probiotics as health-preventive strategies (Rodriguez et al. 2011). In previous studies, the authors of this paper observed that the combination of both additives in extensive ponds, infected with white spot syndrome virus (WSSV, the infectious agent of WSD), significantly increased the yield, although shrimp weight at harvest was low compared with those obtained by the Ecuadorian producers (Rodríguez et al. 2011). Shrimp immunostimulation resulting in a high survival rate (81 %) is, under specific conditions, probably able to provoke intra-specific competition and, as a consequence of higher than foreseen population densities, to reduce shrimp growth and therefore individual weight at harvest.

Intraspecific competition for resources affects the individual weight and population density; therefore, the variables growth and survival/mortality can be used to infer its effect on the yield in aquaculture (Fréchette et al. 2005) and help to determine optimal stocking densities (OSD) (Fréchette et al. 1996, 2000). OSD is defined as the population density at which the maximum yield at harvest is obtained (Fréchette et al. 1996). OSD can also be defined as the stocking density at which maximum economic returns at harvest are obtained. However, the two definitions can give different results, as maximum yield does not necessarily imply maximum profits (Jolly and Clonts 1993). The harvested biomass depends on population density and average individual weight at harvest. Therefore, harvest should be carried out at such a culture time when the combination of both variables has only just started to demonstrate intraspecific competition for growth (beginning of growth reduction). Optimal population density during the whole production cycle should also be at a level such that it does not lead to more intense competition (density-dependent mortalities) or provokes mortalities explained by other density-dependent factors, such as infectious diseases. These parameters can be inferred through the analysis of biomass (B) and population density (N) variations during the production cycle, by the use of the

B–N graphs, which illustrate the curves of biomass variation versus population density, with data obtained from the experiments at various times and stocking densities (Fréchette et al. 1996).

In spite of the apparent importance of the role of intraspecific competition affecting shrimp pond production at low densities, the densities at which competition for growth and survival takes place is not known. Therefore, it becomes necessary to improve our knowledge of density-dependent factors, such as intraspecific competition, affecting shrimp ponds. This would include pond populations where health-preventive treatments are currently used to increase shrimp survival. A combination of knowledge about effective health strategies and optimal population densities could improve disease prevention strategies and maximize the production and profit in extensive/semi-intensive shrimp ponds.

The main objective of this study was to determine whether intraspecific competition takes place in extensive/semi-intensive ponds by comparing the production and economic variables of the *Penaeus vannamei* shrimp, during the experimental culture of healthy ponds stocked at various densities. Specifically, we used information of estimated production variables during the experimental culture, and production and economic variables registered at harvest in order to (1) estimate an optimal shrimp density, through the inference of the density at which competition for growth took place and (2) determine optimal stocking densities, through the selection of the stocking density treatment that, achieving maximum yield (biomass), shows evidence that the beginning of the biomass stagnation occurred near the harvest, and therefore, best economic returns are obtained at harvest.

Materials and methods

Experimental setup

The experiment was designed to compare shrimp production and economic performance of ponds stocked at 3, 6, 9, and 12 shrimp m^{-2}. Stocking densities used in the experimental culture were in the same range as extensive/semi-intensive densities used by Ecuadorian shrimp farmers, whose large environment-dependent ponds are managed without aeration and limited feed. Four ponds (= replicates) were used for each stocking density level, and treatments were randomly allocated to ponds. All ponds were stocked with *P. vannamei* postlarvae PL12 (12 days postlarvae) (1.62 ± 0.01 mg). The experiment was carried out during the wet/warm season (October 19, 2010–March 1, 2011; 133 days).

Shrimp source and experimental ponds

Nauplii 5 (N5) of *P. vannamei* were obtained from Aquatropical hatchery (Mar Bravo, Santa Elena Province, Ecuador) and transported to the research facilities of the Centro Nacional de Acuicultura e Investigaciones Marinas (CENAIM, 187 km from Guayaquil, Ecuador). Shrimp were reared until PL12. The experiment was carried out in sixteen 400 m^2 earthen ponds of the CENAIM experimental station located in Palmar 170 km NW of Guayaquil, Ecuador (2°00′51.40″S-80°43′21.17″W). Prior to stocking, the ponds were dried for at least 10 days and filled with pumped seawater of 37 g L^{-1} from the nearby sea. Water depth was maintained at about 0.8 m during the whole experiment through some water exchanges in order to recover the water level for evaporation and filtration.

Feeding protocol

For the first 2 weeks of culture period, shrimp were fed daily with a commercial 35 % crude protein sinking pelleted feed (EXPALSA) and afterward until harvest fed with a similar brand containing 28 % crude protein (EXPALSA). The food was distributed manually and supplied twice daily (07:30 and 15:00; the proportion of the total fed at each feeding was 50 %) starting at 12 % of their initial biomass and adjusted gradually to 2.5 % at the end of the culture. This feeding regime was alike for all replicates.

Health-preventive treatments

Preventive treatments currently used in CENAIM and by Ecuadorian producers to enhance the population health at pond level (Rodríguez et al. 2007, 2011) were used during the larviculture and grow-out phases. This strategy aimed to remove the effect of diseases on the population densities in the experimental units, by reducing the risk of disease outbreaks. In this way, the effect of shrimp density on competition for space and food was isolated.

Preventive health treatments in the larviculture phase

In the early larviculture phase (N5-PL4), *Vibrio alginolyticus* (Ili strain) probiotic was added daily to the culture water, following the protocol described by Rodríguez et al. (2007). This strain was isolated originally from healthy shrimp larviculture (Morales, unpublished data) and is commonly used in CENAIM's larviculture protocol to mitigate what is commonly referred to as "bolitas" syndrome or Zoea 2 syndrome (Vandenberghe et al. 1999). β-1,3/1,6-glucans (extracted from *Saccharomyces cerevisiae* walls) were administered from PL4 to PL12 to the formulated diet, following the protocol described by Rodríguez et al. (2011).

Preventive health treatments in the grow-out phase

Commercial feed was supplemented with β-1,3/1,6-glucans at the start of feeding, according to the protocol described in Rodríguez et al. (2011). In addition, two probiotic strains were provided via food at a rate of 1 ml kg^{-1} of each strain, with a final concentration of 1×10^3 CFU g^{-1}.

Data collection

Physical and chemical variables

Water temperature (mercury thermometer) and dissolved oxygen concentration adjusted for salinity (YSI 55 dissolved oxygen meter—YSI Incorporated, Ohio, USA) were registered daily in situ at 06:00 in each replicate.

Estimated production variables during the experimental culture

From day 55, shrimp average fresh weight (g) was estimated weekly in each replicate through cast net samplings. Shrimp samples ($n > 80$ shrimp) were weighed using a

precision scale of 0.1 g (AandD Company Limited, Korea) and divided by the number of shrimp.

Shrimp density was estimated through cast nest samplings (monofilament net, 5 m^2 of opening area) at days 76 and 99, using the average of the number of shrimp per cast net area (4 casts for each pond). Survival and biomass at both dates were estimated using the information of shrimp density and weekly shrimp average weight.

Production and economic variables registered at harvest

At 133 days of culture, the harvest biomass (kg) per pond was registered in each replicate to calculate the production variables. A random sample of 100 shrimp was collected in each replicate to estimate the shrimp average weight (g). The total amount of food (kg) supplied throughout the production cycle was also calculated for each replicate. Using this information, the following variables were determined in each replicate: *yield* (kg ha^{-1}), calculated as the harvest biomass divided by the pond area; the *number of harvested shrimp*, calculated as the harvest biomass divided by the shrimp average weight; *survival*, calculated as the number of harvested shrimp divided by the number of stocked shrimp, expressed as a percentage; the *harvest density* (shrimp m^{-2}), calculated as the number of harvested shrimp divided by the pond area and the feed conversion rate (*FCR)*, dimensionless parameter calculated as the total amount of feed (formulated) supplied throughout the production cycle divided by the harvest biomass.

For the economic analysis, the following variables were calculated for each replicate: (1) *total production cost* (in dollars), determined using the costs of the stocked larvae and the total amount of feed supplied throughout the production cycle. For the larvae cost, the market value at the stocking time (US$ 1.35 per 1,000 larvae) was used. For the food cost, differential costs were determined in accordance with the protein percentage; so the costs of commercial feed with 28 and 35 % protein were 0.70 and US$ 0.93 kg^{-1}, respectively. Some costs such as labor costs, pond investments, among others were not taken into account as they did not reduce the net revenue; (2) the *gross receipt* (in dollars) was calculated using the shrimp market price. The harvested shrimp were selected by size, and using the classification code prices, the value of the shrimp was calculated. Prices were provided by a commercial exporter plant in Ecuador (Table 1); (3) the *net return* (in dollars) was calculated as the difference between total production costs and sales; (4) the *benefit–cost rate* was determined as the ratio between sales and total production costs, which represents the benefits provided by each dollar spent during the production cycle. The incremental cost by using β-1,3/1,6-glucans and probiotics in the health-preventive treatments for larviculture and grow-out phases was not added in the calculations due to the difference in costs between treatments being negligible.

Data analysis

Physical and chemical variables

Temperature and dissolved oxygen concentration were registered each morning and averaged on a weekly basis. Weekly data of dissolved oxygen were analyzed through a two-factor (stocking density and time) ANOVA model, with repeated measures on time (levels on 19 weeks). Mauchly's sphericity test was used to examine the form of the common covariance matrix for the factors time and interaction between time and stocking density (Quinn and Keough 2002). This analysis was performed to test whether the matrix

Table 1 Reference prices for April 2011 of head-on shrimp, related to market size (classification code)

Classification code	Shrimp weight minimum (g)	Shrimp weight maximum (g)	Price ($ kg^{-1})
140–200[a]	5.00	7.14	2.95
120–140	7.14	8.33	3.15
100–120	8.33	10.00	3.6
80–100	10.00	12.50	4.2
70–80	12.50	14.29	4.3
60–70	14.29	16.67	4.5
50–60	16.67	20.00	4.6
40–50	20.00	25.00	4.7
30–40	25.00	33.33	6.2

[a] For example, the 140–200 classification code corresponds to a commercial size of between 140 and 200 shrimp per kilo (commercial package of head-on shrimp)

of the variances of the differences between values of the dissolved oxygen were the same for all pair of weeks (sphericity assumption). When significant results were found using the Mauchly test, sphericity was not achieved (meaning that the values of dissolved oxygen were correlated on time), and the degrees of freedom of time and the interaction between time and stocking density of the ANOVA analysis were adjusted multiplying by the Huynh–Feldt epsilon correction factor (degree to which the covariance matrix departs from sphericity).

Estimated production variables during the experimental culture

Slopes of the weekly average of shrimp weight of all four treatments were compared from days 55 (week 8) until harvest at day 133 (week 19), using the F test for differences between regression functions (Zar 1999). Tukey's test for multiple comparisons was used to compare slopes of all four treatments (Zar 1999).

The B–N graph (Westoby 1984) was constructed in order to analyze the variation of biomass and shrimp density, and thereby infer levels of shrimp density and culture time where intraspecific competition occurs (Fréchette et al. 1996, 2000, 2005; Qin et al. 2009). The graph included information of four dates: stocking (0 day), days 76 and 99, and harvest (day 133). The B–N graph was used for both the analyses of trajectories followed by the B–N pairs on time for all replicate of each treatment (3, 6, 9, and 12 shrimp m^{-2}) and analyses of trajectories followed by the B–N pairs on stocking density treatments for each time (days 76, 99, and 133).

One-way ANOVAs were performed at days 76 and 99 to compare among treatments the effects of stocking density on the variables estimated in the population samplings (biomass, individual shrimp weight, and survival). Variance homogeneity of all treatments was examined using Levene's statistic. Assumption of normality was examined through the Kolmogorov–Smirnov test. Based on these results, some variables were transformed to ln $(\times)$ or $1/\times^2$ in order to homogenize the variances. The effect of the factors and differences between treatments were considered significant at $p < 0.05$. When ANOVA showed statistical significance, Tukey's test was used to compare treatment means. Survival at 76 day did not achieve the assumption of variance homogeneity; therefore, differences among treatments were analyzed using a Kruskal–Wallis one-way ANOVA.

Finally, the F test for the lack of fit for a regression with replicates (Neter et al. 1996) was applied to determine whether the linear regression function fitted the estimated data of biomass (dependent variable) to the first three (3, 6, and 9 shrimp m^{-2}) or four levels (3, 6, 9, and 12 shrimp m^{-2}) of the stocking density treatment (independent variable).

Production and economic variables registered at harvest

The effects of stocking density on the production and economic variables registered at harvest (biomass, individual shrimp weight, yield, survival, harvest density, FCR, total costs of production, gross receipt, net return, and benefit–cost rate) were compared among treatments using a one-way ANOVA design. Variance homogeneity of all treatments was examined using Levene's statistic. Assumption of normality was examined through the Kolmogorov–Smirnov test. Based on these results, some variables were transformed to ln (×) in order to homogenize the variances. The effect of the factors and differences between treatments were considered significant at $p < 0.05$. When ANOVA showed statistical significance, Tukey's test was used to compare treatment means.

Treatment averages were expressed as mean ± standard deviation (SD). Statistical analysis was performed with the Statistical Package for Social Sciences software Windows version 11.0 (SPSS, Chicago, IL, USA).

Chi square's goodness-of-fit test (Dickinson 1997) was used to compare the frequencies of occurrences of shrimp sizes between stocking density treatments at harvest using the market size categorization (Table 1). For this analysis, the samples of 100 shrimp randomly selected at harvest in each replicate were used. The frequencies of the three higher commercial sizes (50–60, 40–50, and 30–40) and the three smaller sizes (140–200, 120–140, and 100–120) (Table 1) were taken together to ensure nonzero counts in all possible comparisons between treatments, where, for example, the 50–60 classification code corresponds to a commercial size of between 50 and 60 shrimp per kilo (commercial package of head-on shrimp). The rest of the commercial size categories were maintained as shown in Table 1. The size distributions of shrimp between two stocking treatments were considered different when $p < 0.05$.

Asymmetries of shrimp average weight at harvest were compared between treatments in order to analyze the shape of the graph of the weight distribution.

Estimation of optimal shrimp density

The optimal shrimp density was defined as the shrimp density during the experimental culture at which competition began or at which a marginal intraspecific competition for growth was observed (beginning of growth reduction), without biomass stagnation and before the start of more intense competition (density-dependent mortalities). The optimal shrimp density was inferred through the data analyses performed at days 76, 99, and 133 (slopes of the weekly average of shrimp weight, B–N graph, ANOVA analyses of biomass, weight, and survival/mortality, and biomass linear regression).

Determination of optimal stocking density

In this study, the optimal stocking density was defined as the stocking density treatment that achieved three criteria: (1) maximum yield (biomass) at harvest, (2) beginning of

biomass stagnation near the harvest, and (3) best economic return at harvest (defined as the treatment with the best net return and benefit–cost rate).

Results

Physical and chemical variables

Water temperature showed the same variability and increase over time in all experimental units, from 22.8 ± 0.6 °C (first week) to 28.0 ± 0.4 °C (last week). Stocking density had no significant effect on dissolved oxygen ($p = 0.192$). Dissolved oxygen in all replicates significantly decreased throughout the culture time ($p < 0.001$). Highest (6.0 ± 1.0 mg L^{-1}) and lowest (< 2.0 mg L^{-1}) values were recorded in the second and last week (Fig. 1). No significant interactions between time and stocking density were found ($p = 0.589$).

Estimated production variables during the experimental culture

Average weekly growth rate was 1.4 ± 0.4, 1.0 ± 0.3, 0.8 ± 0.3, and 0.8 ± 0.3 g per individual in treatments 3, 6, 9, and 12 shrimp m^{-2}, respectively. Slopes of shrimp weight from weeks 8 till 19 (between days 55 and 133) were significantly different ($p < 0.05$) between 3, 6, and 9 shrimp m^{-2} treatments, but not between 9 and 12 shrimp m^{-2} (Fig. 2).

A biomass increase accompanied by a proportional decrease in shrimp density was observed in replicates of all four treatments from stocking to day 76, suggesting that these mortalities (≈ 19 %) could be caused by a density-independent factor (Fig. 3). Between days 76 and 99, stocked ponds up to 9 shrimp m^{-2} had an increase in biomass without major changes in the shrimp density and therefore did not affect the survival; on the contrary, ponds stocked at 12 shrimp m^{-2} showed in general a biomass increase accompanied by a decrease in shrimp density (Fig. 3). Between days 99 and 133, all replicates showed a biomass increase without major changes in shrimp density, except in one pond of the 12 shrimp m^{-2} treatment (Fig. 3).

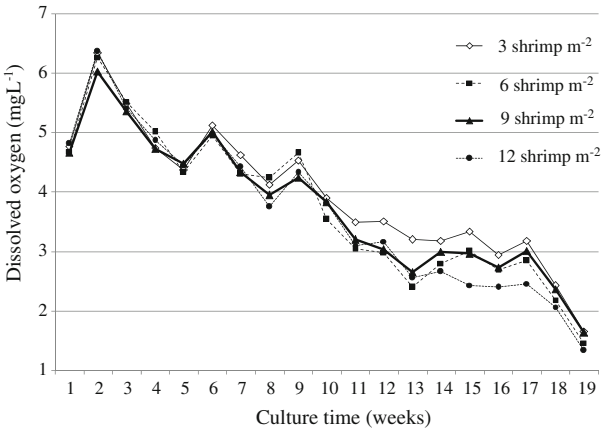

Fig. 1 Dissolved oxygen concentration (mg L^{-1}) in experimental ponds stocked at 3, 6, 9, and 12 shrimp m^{-2}

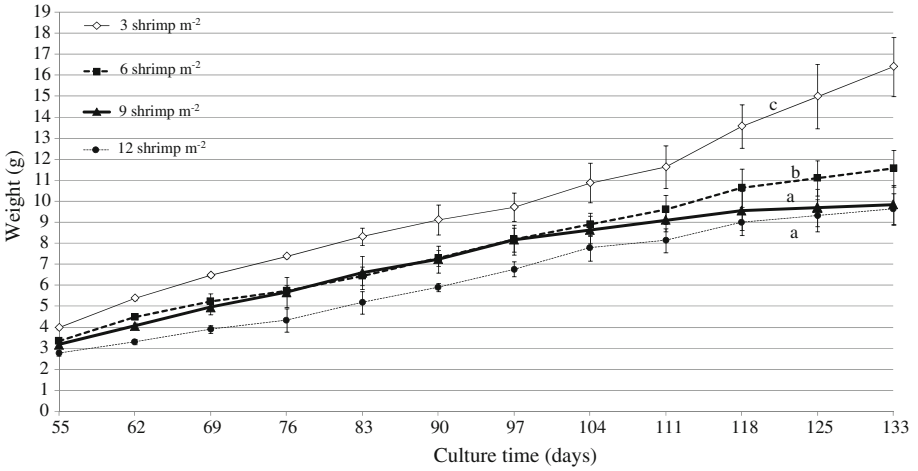

Fig. 2 Average of individual shrimp weight (g) in experimental ponds stocked at 3, 6, 9, and 12 shrimp m^{-2}. Data are expressed as mean ± SD. In each weekly growth curve, *different letters* indicate linear regression functions significantly different at $p < 0.05$

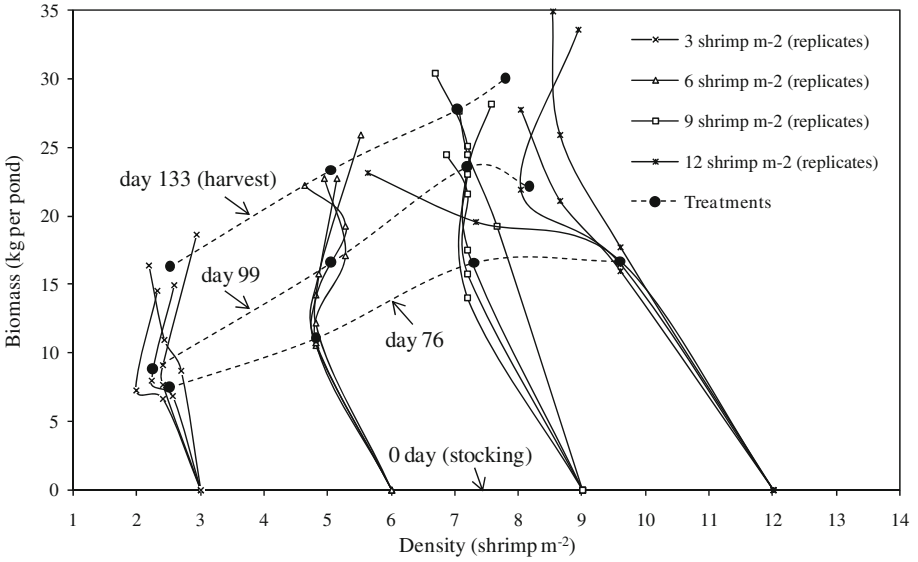

Fig. 3 Biomass (kg) per pond—shrimp density (shrimp m^{-2}) (B–N graph) of the replicates of four stocking density treatments (3, 6, 9, and 12 shrimp m^{-2}). The *solid lines* represent the variation of biomass–density pairs for each one of the replicates, starting from stocking (day 0) and ending at harvest (day 133). The biomass–density pair intermediate values were estimated with population samplings at days 76 and 99. The dashed lines link the average of biomass–density values of all four treatments at days 76, 99, and 133 (harvest), respectively

Significant differences ($p < 0.002$) of estimated biomass between ponds stocked at 3, 6, and 9 shrimp m^{-2} were found at days 76 and 99, but not between 9 and 12 shrimp m^{-2} ($p > 0.755$, Table 2). Shrimp weight stocked at 3 shrimp m^{-2} was significantly higher ($p < 0.046$) compared to all other three treatments at days 76 and 99 (Table 2). At day 76,

Table 2 Effect of stocking density on biomass, individual shrimp weight, and survival at days 76 and 99

Estimated variables	Stocking density treatments (shrimp m^{-2})	Culture day	
		Day 76	Day 99
Biomass (kg) per pond	3	7.4 ± 0.9a	8.8 ± 1.6a
	6	11.0 ± 0.8b	16.6 ± 2.1b
	9	16.6 ± 2.2c	23.5 ± 1.6c
	12	16.7 ± 0.7c	22.1 ± 2.7c
Individual shrimp weight (g)	3	7.4 ± 0.7c	9.7 ± 1.0b
	6	5.7 ± 0.4b	8.2 ± 0.7a
	9	5.7 ± 0.6b	8.2 ± 0.5a
	12	4.3 ± 0.2a	6.8 ± 0.6a
Survival (%)	3	83.8 ± 4.8a	75.3 ± 6.9ac
	6	80.0 ± 0.0a	84.3 ± 4.3bc
	9	81.3 ± 2.5a	80.0 ± 0.0bc
	12	80.0 ± 0.0a	68.0 ± 5.2a

Data are expressed as mean ± SD. In each column and for each variable, averages indicated by different letters are significantly different at $p < 0.05$, based on one-way analysis of variance and Kruskal–Wallis one-way analysis of variance (survival at day 76)

shrimp from ponds stocked at 12 shrimp m^{-2} showed significantly less weight than those of other treatments ($p < 0.004$); shrimp weights stocked at 6 and 9 shrimp m^{-2} were similar ($p = 0.992$), but significantly lower than 3 at 76 and 99 days ($p < 0.006$), and higher than 12 shrimp m^{-2} treatments at 76 days ($p < 0.004$) (Table 2). Survival was not affected by stocking density at day 76 ($p = 0.222$), but a stocking density effect on survival was observed at day 99, with lower survival in ponds stocked at 12 shrimp m^{-2} ($p < 0.024$) (Table 2) compared with the 6 and 9 shrimp m^{-2} treatment.

At days 76 and 99, the estimated biomass increased linearly ($p < 0.05$) with stocking density at the first three stocking levels, while at day 133 a linear relationship ($p < 0.05$) was found at all four levels of stocking density (Fig. 3; Table 2).

Production and economic variables registered at harvest

Biomass and yield of ponds stocked at 3 shrimp m^{-2} were significantly lower compared with the other three treatments ($p < 0.005$), between which no significant differences were found ($p > 0.083$). Survival in ponds stocked at 3, 6, and 9 shrimp m^{-2} were not significantly different ($p > 0.791$). However, shrimp survival of ponds stocked at 6 shrimp m^{-2} was significantly higher compared with ponds stocked at 12 shrimp m^{-2} ($p < 0.047$). Shrimp survival of ponds stocked at 12 shrimp m^{-2} showed no significant difference compared with ponds stocked at 3 and 9 shrimp m^{-2} ($p > 0.057$). Harvest density was significantly different between ponds stocked at 3, 6, and 9 shrimp m^{-2} ($p < 0.020$). But, no significant differences were found between ponds stocked at 9 and 12 shrimp m^{-2} ($p = 0.582$). Shrimp from ponds stocked at 3 shrimp m^{-2} showed a significantly higher individual weight compared with the other three treatments ($p < 0.001$), while among the latter no significant differences were found ($p > 0.140$). FCR of shrimp stocked at 3 and 6 shrimp m^{-2} was less than 1 and significantly lower than for shrimp stocked at 12 shrimp m^{-2} ($p < 0.002$). No significant differences of FCR were found between ponds stocked at

9 and 12 shrimp m^{-2} ($p = 0.161$), nor between ponds stocked at 6 and 9 shrimp m^{-2} ($p = 0.104$).

Total production cost increased significantly and was directly proportional to the stocking density (Table 3). Gross receipt obtained in ponds stocked at 6, 9, and 12 shrimp m^{-2} were not significantly different among themselves ($p > 0.553$), and no significant differences were reported between ponds stocked at 3 and 6 shrimp m^{-2} ($p = 0.054$). Net return was not significantly associated with stocking density ($p = 0.448$). Benefit–cost rate of treatments indicated a significant greater monetary return in ponds stocked at 3 and 6 shrimp m^{-2} ($p < 0.042$). No significant differences were obtained between ponds stocked at 3 and 6 shrimp m^{-2} ($p = 0.078$) or between 9 and 12 shrimp m^{-2} treatments ($p < 0.210$).

The commercial size distribution was different in all treatments, except between ponds stocked at 9 and 12 shrimp m^{-2} ($p > 0.05$). The asymmetries of shrimp average weight at harvest were similar (0.49 ± 0.12) at 9 and 12 shrimp m^{-2}. Lowest asymmetry was observed at 6 shrimp m^{-2} (0.04 ± 0.12). Asymmetry at the 3 shrimp m^{-2} treatment was 0.23 ± 0.12.

Estimation of optimal shrimp density

The previous results suggested that shrimp from 6, 9, and 12 shrimp m^{-2} treatments presented competition-dependent growth during the whole culture period, especially at 9 and 12, and more intensely at 12 shrimp m^{-2} (Tables 2, 3; Figs. 2, 3). Biomass stagnation occurred between days 99 and 133 for the 6 and 9 shrimp m^{-2} treatments, with the 9 shrimp m^{-2} treatment suffering more. For the 12 shrimp m^{-2} treatment, the biomass stagnation may have begun at day 76 or even earlier. Competition, and accordingly individual growth reduction, may have begun at day 76 at a density of 5 shrimp m^{-2}

Table 3 Effect of stocking density on the production and economic variables (mean ± SD) in experimental ponds (400 m^2) obtained at harvest (day 133)

Variable	Stocking density (shrimp m^{-2})			
	3	6	9	12
Biomass (kg) per pond	16.1 ± 1.8a	23.4 ± 1.7b	27.7 ± 2.4b	29.9 ± 5.4b
Harvest density (shrimp m^{-2})	2.5 ± 0.3a	5.1 ± 0.4b	7.0 ± 0.4c	7.8 ± 1.5c
Individual shrimp weight (g)	16.2 ± 1.8b	11.6 ± 0.4a	9.8 ± 1.1a	9.6 ± 0.8a
Survival (%)	83.5 ± 11.3ab	84.2 ± 6.2b	78.2 ± 4.2ab	64.8 ± 12.4a
Yield (kg ha^{-1})	402.6 ± 45.8a	584.0 ± 41.9b	691.7 ± 60.7b	746.0 ± 136.3b
Feed conversion rate	0.81 ± 0.12a	0.97 ± 0.04ac	1.25 ± 0.15bc	1.57 ± 0.29b
Total production cost ($ per 400 m^2)	10.79 ± 0.43a	19.48 ± 0.58b	29.49 ± 0.98c	39.20 ± 0.62d
Gross receipt ($ per 400 m^2)	72.85 ± 8.68a	97.55 ± 7.34ab	106.25 ± 15.67b	113.19 ± 22.67b
Net return ($ per 400 m^2)	62.07 ± 9.11a	78.07 ± 6.85a	76.75 ± 16.50a	74.00 ± 22.31a
Benefit–cost rate ($ per 400 m^2)	6.79 ± 1.09b	5.00 ± 0.25b	3.62 ± 0.65a	2.88 ± 0.55a

In each row, the averages indicated by *different letters* are significantly different at $p < 0.05$, based on one-way analysis of variance analyses

Table 4 Performance of the stocking density treatments based on three criteria achieved during the experimental culture and harvest

Criterion	Stocking density (shrimp m^{-2}) (Harvest density) (shrimp m^{-2})			
	3 (2.5 ± 0.3)	6 (5.1 ± 0.4)	9 (7.0 ± 0.4)	12 (7.8 ± 1.5)
Maximum yield (biomass) at harvest		+	+	+
Beginning of the biomass stagnation at harvest		+		
Best economic return at harvest				
Best net return	+	+	+	+
Best benefit–cost rate	+	+		

The symbol plus (+) means that the criterion was achieved

Harvest densities are shown in parentheses

(shrimp density average in ponds stocked at 6 shrimp m^{-2} at days 76 and 99) and maintained until harvest. At day 133, ponds stocked at 6 shrimp m^{-2} maintained the same condition as observed at day 99 (the absence of mortalities), but biomass reached similar values to those reported in ponds stocked at 9 and 12 shrimp m^{-2}. Consequently, optimal shrimp density during the experimental culture was 5 shrimp m^{-2}.

Determination of optimal stocking density

The maximum yield (biomass) at harvest was achieved with ponds stocked at 6, 9, and 12 shrimp m^{-2} (without statistical differences) (Table 4). The beginning of biomass stagnation near the harvest was observed in ponds stocked at 6 shrimp m^{-2} (Table 4). Similar best net return were obtained in all four treatments (without statistical differences); however, the best benefit–cost rates were obtained with ponds stocked at 3 and 6 shrimp m^{-2} (Table 4). Based on these results, it was observed that only ponds stocked at 6 shrimp m^{-2} achieved the three criteria defined as the optimal stocking density (Table 4).

Discussion

The results of this study provide information to optimize the management in extensive/semi-intensive shrimp farming systems. Our findings support the results of the Ecuadorian shrimp producers who, based on empirical observations, have generated the recovery of the cultured shrimp industry after the WSD epidemic by decreasing the stocking densities.

In our experiment, dissolved oxygen concentrations decreased during the production cycle, in spite of several water exchanges that were performed. Salinity increased during the production cycle (up to 42 g L^{-1}), which in combination with the increased water temperature (5.2 °C increment during the production cycle) could provoke lower solubility of oxygen and present levels of oxygen saturation at about 32 % at the last culture week (when the environmental conditions were 28 °C, 2 mg L^{-1} of dissolved oxygen, 40 g L^{-1} of salinity, and assuming 760 mm Hg of air pressure). This could provoke stress conditions for shrimp as, in addition at high salinities and temperatures, the metabolic demand of *P. vannamei* rises due to the effort required to maintain the osmotic and ionic balance

(Villarreal et al. 1994). Up to day 76, the levels of dissolved oxygen were in general above 3 mg L^{-1}; therefore, a too low oxygen concentration neither explained the lower increase in individual shrimp weight of ponds stocked at 6, 9, and 12 shrimp m^{-2}, nor the mortalities observed in all four treatments at day 76. However, between weeks 13 and 18, oxygen levels were lower than 3 mg L^{-1} in ponds stocked at 6, 9, and 12 shrimp m^{-2}, which may be density-related and might contribute to growth reduction in those treatments. During the last week of production, all ponds presented dissolved oxygen values less than 2 mg L^{-1}, which could affect the shrimp growth in all ponds.

Growth reduction in isolated lobsters *Homarus americanus*, *H. gammarus,* and *Macrobrachium rosenbergii* could be chemically mediated by the generation of metabolites causing growth retardation (Nelson et al. 1980; D'Abramo et al. 2000). Also, the response of growth retardation by an apparent chemical stimulus, even though it seems be ephemeral (Nelson et al. 1980), seems occur instantaneously (D'Abramo et al. 2000), when a biomass threshold is achieved. Although this effect has been observed in other decapods at laboratory level, and the volume of shrimp ponds is much higher, it is possible that this effect has provoked or contributed to the growth and biomass reduction observed in our experimental culture at higher shrimp densities.

Space has been attributed as a factor causing growth reduction in crustaceans (Maguire and Leedow 1983; Peterson and Griffith 1999). Shrimp density can increase in particular areas of the pond in critical environments, resulting in crowding, which in turn could cause density-dependent problems such as mortality or lack of growth. Shrimp can avoid hypoxic regions (Renaud 1986) and also select welfare temperatures (Hernández et al. 2006). We hypothesize that is also possible that competition for space, in order to find the best pond zones, especially for dissolved oxygen, could also have occurred in our experiment.

Feed conversion rate less than 1 was registered in our ponds stocked at 3 and 6 shrimp m^{-2}, indicating that there was proportionally a good contribution of the natural productivity. FCR higher than 1 were registered in ponds stocked at 9 and 12 shrimp m^{-2} (harvest densities > 7.0 shrimp m^{-2}), which does not necessarily suggest a benthos depletion or an inefficient replacement of the natural food by the formulated food during the production cycle, as if there is a biomass threshold that limits growth; then, there will be increases in FCR unrelated to the availability of food resources.

Optimal shrimp density during the experimental culture was 5 shrimp m^{-2}, which was the harvest density of ponds stocked at 6 shrimp m^{-2}. Considering the fraction of the density-independent mortality reported at day 76 in this treatment (with similar values for all other three treatments), the optimal stocking density taking into account such overstocking was about 6 shrimp m^{-2}. In the absence of density-independent mortality, the optimal stocking density would be 5 shrimp m^{-2}. It is interesting to note that Ecuadorian shrimp farmers obtain in average harvest densities between 4 and 5 shrimp m^{-2}, using stocking densities between 8 and 10 shrimp m^{-2}.

Intraspecific competition has been described for both natural and aquaculture populations (Fréchette et al. 1996, 2000; Guiñez 2005; Qin et al. 2009), including crustaceans (Morrissy 1992), and has been attributed to the competition for food and space. When the biomass threshold is already stagnant in a population, highest levels of competition can occur causing mortality and consequent biomass reduction above the threshold level; such mortality is called self-thinning (Lachance-Bernard et al. 2010). Although we found significant differences of survival at harvest between ponds stocked at 6 and 12 shrimp m^{-2}, no differences were found between 3 and 12 shrimp m^{-2} treatments; therefore, we cannot attribute that the mortalities in the last treatment were provoked by a self-thinning process. Other studies have no reported association between shrimp survivals and stocking density

(Wyban et al. 1987; Allan and Maguire 1992; Yip-Hoi 2003). Our results showed density-dependent growth in the culture of *P. vannamei*. However, additional studies that include intensive densities will be necessary, to study whether the self-thinning process takes place in *P. vannamei* production.

The results of this experiment showed that stocking density had a significant effect on *P. vannamei* shrimp production, with some advantages to stocking at 6 shrimp m^{-2} (survival, FCR, and benefit–cost rate at 6 shrimp m^{-2} were significantly better compared to ponds stocked at 12 shrimp m^{-2}). Stocking at 6 shrimp m^{-2} was enough to reach similar levels of shrimp weight and yield obtained by stocking even with 12 shrimp m^{-2}. Stocking at 6 shrimp m^{-2} would be a safer investment due to a lower variability of biomass, individual weight, net return, and benefit–cost rate, in contrast to the 12 shrimp m^{-2} treatment. These observations were indicative of a good performance of these ponds.

Optimal stocking and shrimp densities could, among others things, depend on particular conditions of each farm/pond, such as soil and food quality, natural productivity, climate, and particular management. However, once they are determined, production costs can be optimized. Our results can be used to maximize the production in extensive/semi-intensive systems and to improve the strategies of disease prevention (such as WSD) in immunostimulated shrimp.

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References

Allan GL, Maguire GB (1992) Effects of stocking density on production of *Penaeus monodon* Fabricius in model farming ponds. Aquaculture 107:49–66

D'Abramo L, Daniels WH, Gerard PD et al (2000) Influence of water volume, surface area, and water replacement rate on weight gain of juvenile freshwater prawns, *Macrobrachium rosenbergii*. Aquaculture 182:161–171

Dickinson J (1997) Nonparametric methods for quantitative analysis, 3rd edn. American Sciences Press Inc, Columbus

Fréchette M, Bergeron P, Gagnon P (1996) On the use of self-thinning relationships in stocking experiments. Aquaculture 145:91–112

Fréchette M, Gaudet M, Vigneau S (2000) Estimating optimal population density for intermediate culture of scallops in spat collector bags. Aquaculture 183:105–124

Fréchette M, Alunno-Bruscia M, Dumais JF et al (2005) Incompleteness and statistical uncertainty in competition/stocking experiments. Aquaculture 246:209–225

Guinez R (2005) A review on self-thinning in mussels. In: Revista de biología marina y oceanografía 40:1–6. Available from http://www.scielo.cl/scielo.php?script=sci_arttext&pid=S0718-19572005000100001&lng=en&nrm=iso. Cited 15 Nov 2012

Hernández M, Buckle LF, Palacios E et al (2006) Preferential behavior of white shrimp *Litopenaeus vannamei* (Boone 1931) by progressive temperature-salinity simultaneous interaction. J Therm Biol 31:565–572

Jolly CM, Clonts HA (1993) Economics of aquaculture. The Haworth Press Inc, Binghamton

Lachance-Bernard M, Daigle G, Himmelman JH et al (2010) Biomass-density relationships and self-thinning of blue mussels (*Mytilus* spp.) reared on self-regulated longlines. Aquaculture 308:34–43

Maguire GB, Leedow MI (1983) A study of the optimum stocking density and feed rate for school prawns *Metapenaeus macleayi* (Haswell) in some Australian brackish water farming ponds. Aquaculture 30:285–297

Morrissy N (1992) Density-dependent pond growout of single year-class cohorts of a freshwater crayfish Cherax tenuimanus (Smith) to two years of age. J World Aquacult Soc 23:154–168

Nelson K, Hedgecock D, Borgeson W et al (1980) Density-dependent growth inhibition in lobsters, *Homarus* (Decapoda, Nephropidae). Biol Bull 159:162–176

Neter J, Kutner MH, Nachtsheim CJ et al (1996) Applied linear statistical models, 4th edn. WCB/McGraw-Hill, Boston

Peterson JJ, Griffith DRW (1999) Intensive nursery systems. Global Aquac Advocate 2:60–61

Qin C, Dong S, Tan F et al (2009) Optimization of stocking density for the sea cucumber, *Apostichopus japonicus* Selenka, under feed-supplement and non-feed-supplement regimes in pond culture. J Ocean Univ China 8:296–302

Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press, New York

Renaud ML (1986) Detecting and avoiding oxygen deficient sea water by brown shrimp, *Penaeus aztecus* (Ives), and white shrimp *Penaeus setiferus* (Linnaeus). J Exp Mar Biol Ecol 98:283–292

Rodríguez J, Espinosa Y, Echeverría F et al (2007) Exposure to probiotics and β-1,3/1,6 glucans in larviculture modifies the immune response of *Penaeus vannamei* juveniles and both the survival to white spot Syndrome Virus challenge and pond culture. Aquaculture 273:405–415

Rodríguez J, Cedeño R, Bayot B et al (2011) Effects of Probiotics, β1,3/1,6-glucans and temperature on shrimp production. In: Russo R (ed) Aquaculture 1. Dynamic Biochemistry, Process Biotechnology and Molecular Biology 5:49–55

Stern S, Sonnenholzner S (2011) Semi-intensive shrimp culture: the history of shrimp farming in Ecuador. In: Alday-Sanz V (ed) The shrimp book. Nottingham University Press, Nottingham, pp 207–232

Vandenberghe J, Verdonck L, Robles-Arozarena R et al (1999) Vibrios associated with *Litopenaeus vannamei* larvae, postlarvae, broodstock, and hatchery probionts. Appl Environ Microbiol 65:2592–2597

Villarreal H, Hinojosa P, Naranjo J (1994) Effect of temperature and salinity on the oxygen consumption of laboratory produced *Penaeus vannamei* postlarvae. Comp Biochem Phys A 108:331–336

Westoby M (1984) The self-thinning rule. Adv Ecol 14:167–225

Wyban JA, Lee CS, Sato VT et al (1987) Effect of stocking density on shrimp growth rates in manure-fertilized ponds. Aquaculture 61:23–32

Yip-Hoi TA (2003) An investigation of effects of dissolved oxygen level, sediment type, stocking density and predation on the growth rate, survivorship, and burrowing behavior of juvenile brown and white shrimp. Dissertation, North Carolina State University, Raleigh, North Carolina

Zar JH (1999) Biostatistical analysis, 4th edn. Prentice Hall Inc, Upper Saddle