

Effect of Stocking Density on Shrimp Growth Rates in Manure-Fertilized Ponds

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ABSTRACT

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Ecuadorian *Penaeus vannamei* were cultured in eight 200-m² dirt ponds at four stocking densities (5, 10, 15 and 20 shrimp/m²). No commercial feed was given to the shrimp. The only input to the ponds was about 36 kg of feedlot cattle manure per pond per week (1800 kg manure ha⁻¹ week⁻¹).

Shrimp growth was not correlated with variations in water quality among treatments (e.g., temperature, pH, DO, secchi visibility). Water-column nutrient levels were less than or equal to nutrients in incoming water and did not increase with addition of cattle manure. Water ATP concentrations were correlated with shrimp stocking density while numerous other biochemical parameters were not. No coliform bacteria were detected in any pond water samples during the study period. Phytoplankton densities and species composition were not different among treatments but varied over time. Two diatoms were the dominant species throughout the study.

There was a negative correlation between stocking density and growth. Mean (\pm SD) weekly shrimp growth across treatments was 0.68 ± 0.00 , 1.06 ± 0.02 , 1.6 ± 0.1 , 1.72 ± 0.2 g individual⁻¹ week⁻¹ for densities of 20, 15, 10, and 5 shrimp/m², respectively. Survival averaged $70.8 \pm 6.3\%$ for all ponds and was not different among treatments. Shrimp production was 19.3 ± 0.1 , 23.0 ± 0.4 , 22.8 ± 1.2 , 12.3 ± 1.1 kg ha⁻¹ day⁻¹ for densities 20, 15, 10, and 5 shrimp/m², respectively. Shrimp production and carrying capacity were not significantly different among the three higher stocking densities.

INTRODUCTION

There are two ways to deliver feeds in aquaculture. One method, which mimics terrestrial animal husbandry, uses formulated feeds. Although effective in terrestrial production, this approach does not transfer well to aquatic systems because many of the essential nutrients contained in pelleted feeds are water soluble and begin leaching out when put in water. Also, these feeds are generally expensive and may not be consumed by the cultured animals. Schroeder (1980) demonstrated that half the growth of common carp in a polyculture

system was based on natural foods found on pond bottom and banks, even in the presence of a full ration of enriched feed pellets. Growth of tilapia in the same system was based 70% on natural foods, and prawns relied almost completely on natural productivity.

In an alternative approach practiced in China and Southeast Asia, nutritive materials are grown in ponds along with cultured animals. It is well known that the addition of manure to fish ponds stimulates heterotrophic productivity which in turn leads to increased fish production (Tang, 1970; Moav et al., 1977; Schroeder, 1978). Most of this work has been done in freshwater pond systems however, and little is known about the effects of manure fertilization in marine ecosystems.

Marine shrimp farming in Hawaii and the U.S.A. relies on commercial diets. Although Lee and Shleser (1984) reported rapid growth of shrimp in ponds fertilized with cattle manure, information on shrimp growth in fertilized ponds without feeds is limited and a negative response to fertilization has been suggested (Rubright et al., 1981; Garson et al., 1984).

Disposal of feedlot manure is a problem for the cattle industry. This manure represents an under-utilized resource if it can be used to produce a valuable crop. This experiment was designed to determine the effects of shrimp stocking density on water quality, certain pond ecosystem biochemical parameters, phytoplankton dynamics, and shrimp growth rate and production in earthen seawater ponds fertilized with cattle manure.

MATERIALS AND METHODS

Ecuadorian *Penaeus vannamei* were stocked in eight 200-m² earthen ponds at densities of 5, 10, 15 and 20 shrimp/m². Each stocking density was replicated in two ponds supplied with seawater from a well at approximately 20% exchange/day. The experiment began on 22 August 1984, and shrimp were harvested on 12 November 1984. Initial average weight of the shrimp was 2.7 g each. No commercial feed was supplied at any time. Feedlot cattle manure was added once weekly to each pond (36 kg pond⁻¹ week⁻¹; 1800 kg ha⁻¹ week⁻¹). This dose had been determined optimal in earlier experiments (Lee and Shleser, 1984). The total amount of manure supplied to each pond through the growout period (11 weeks) was 400 kg.

Water quality parameters (temperature, pH, DO and turbidity) were recorded twice daily (09.00 h and 16.00 h) for each of the ponds. Water chemistry and floristics were monitored weekly in four ponds (5, 6, 7, 8) representing each stocking density. Nitrate and nitrite nitrogen, ammonium and phosphate levels in the ponds and in manure were measured twice weekly with an auto-analyzer (Strickland and Parsons, 1972). Carbon and nitrogen composition of the manure was determined with a CHN auto-analyzer (Hewlett-Packard Model 185 Carbon-Hydrogen-Nitrogen Analyzer). Sediment hydro-

gen sulphide (H_2S), sediment and suspended adenosine triphosphate (ATP), phytoplankton and zooplankton were measured in four ponds weekly. Sediment H_2S was measured with an Orion sulfide ion electrode (Model 94-16). Water ATP was monitored with the method developed by Karl (1978) and sediment ATP measurement followed the method of Karl and Craven (1980).

Weekly water samples were analyzed for coliform using APHA (1975) methods. Following harvest, shrimp samples were supplied to Dr. James Brock, the State of Hawaii's Aquatic Veterinarian, for bacteriological examination.

Samples for phytoplankton and zooplankton monitoring were collected during the day on a weekly basis in 250-ml dark Nalgene bottles and counted fresh or preserved in 10% formalin in seawater. Organisms were identified to the genus level. Phytoplankton taxa were ranked by relative abundance and relative frequency. Relative abundance was calculated as the total cell density of a single taxon counted throughout the study divided by the grand total cell density. Relative frequency was calculated as a count of the number of samples in which a taxon occurred divided by the total number of observations. Benthic sediment samples were collected with a 2.2-cm diameter core tube. Surface layers were surveyed for microscopic flora and fauna.

Shrimp growth was determined by weekly sampling of 25 individuals/pond which were returned to their ponds after weighing. Instantaneous growth rates were used to estimate carrying capacity (Hepher and Pruginin, 1981). Weight at zero growth was determined by plotting instantaneous growth (weight gain/week) against shrimp size at the beginning of the week for different stocking densities. By multiplying the weight at zero growth (x -intercept) by the stocking density, an approximation of carrying capacity was derived for the four stocking densities used in this experiment.

Treatment means for growth, production, survival, and chemical parameters were compared by ANOVA. Separation of means that differed significantly was performed using Student-Newman-Keuls test (Sokal and Rohlf, 1969). In week 10, a sink hole developed in pond 3 with 15 shrimp/ m^2 and all shrimp were lost. Due to this loss, data for the 15 shrimp/ m^2 treatment at harvest were derived from a single sample (pond 6). Growth rates, production, and biomass increase were calculated using week 9 data so that statistical analyses could include the 15 shrimp/ m^2 treatment.

RESULTS

Water temperature, pH, DO and secchi visibility in the ponds are listed in Table 1. There was no significant difference among densities for any of these parameters. There were no significant differences in dissolved nutrient concentrations among the treatments throughout the experiment (Table 2). The average composition of nitrogen, phosphorus and potassium in the cattle manure, based on the dried weight, throughout the experiment was $2.36 \pm 0.48\%$,

TABLE 1

Mean, standard deviation (SD) and range in water temperature, pH, DO, and secchi visibility at 09.00h and 16.00 h in all ponds during the 11-week growout period

		09.00 h	16.00 h
Temperature (°C)	Mean	27.0 ^a	30.9 ^b
	SD	1.6	1.7
	Range	21.5-28.5	25.5-35.2
pH	Mean	8.1 ^a	8.4 ^b
	SD	0.1	0.1
	Range	7.1- 8.6	7.1- 9.1
DO (mg/l)	Mean	5.9 ^a	12.7 ^b
	SD	0.5	1.1
	Range	3.2- 9.8	4.8-20.0
Secchi depth (cm)	Mean	72 ^a	66 ^a
	SD	10	9
	Range	30-100	30-100

^{a,b}Mean values across rows with different superscripts were significantly different ($P < 0.05$).

$0.97 \pm 0.45\%$ and $3.72 \pm 1.55\%$, respectively. Sediment H_2S averaged from 0.2 to 0.4 mg/l and was not significantly different among the treatments. Water ATP (wATP) fell within the range 2.8-4.5 $\mu\text{g/l}$ and was significantly correlated ($r^2 = 0.99$, $P < 0.05$) with stocking density. Sediment ATP measurements were mostly between 0.3 $\mu\text{g/g}$ and 0.7 $\mu\text{g/g}$ dry matter. Mean chlorophyll (Chl-a) concentrations ranged from 6.5 to 8.4 $\mu\text{g/l}$ and were not significantly different among treatments. The ratio of Chl-a to wATP ranged from 1.8 to 2.7 and was not significantly different among treatments. Coliform colonies were not detected in any of the water samples and no trace of *Salmonella* sp. was detected in any shrimp examined by the State Veterinarian.

Phytoplankton communities are summarized in Table 3. Two diatoms, *Chaetoceros* sp. and *Rhizosolenia* sp., were the dominant phytoplankton groups in the four ponds throughout the experiment. *Chaetoceros* mean (\pm SD) density over all ponds and weeks was $46\,000 \pm 7200$ cells/ml. *Rhizosolenia* mean was $39\,000 \pm 6100$ cells/ml. Blooms of at least 1000 cells/ml of *Chaetoceros* were present in 93% of the samples analyzed, while *Rhizosolenia* was present at that level in 83% of the samples. In all four ponds studied, *Chaetoceros* was present the first week after filling, while *Rhizosolenia* blooms were not established for several weeks after ponds were filled and stocked. *Chaetoceros* represented 38.9% of the total number of phytoplankton cells counted in the four ponds over 11 weeks. *Rhizosolenia* represented 34.7% of the total number of phytoplankton cells. There were no significant differences among stocking densities for phytoplankton densities within weeks.

TABLE 2

Mean and standard deviation (in parentheses) of nutrient and biochemical concentrations measured weekly in four ponds and incoming water during the 11-week growout period

Density (no./m ²)	Incoming water	5	10	15	20
NO ₃ + NO ₂ (μM)	23.2 ^a	9 ^b (2.4)	0.9 ^b (2.5)	0.6 ^b (1.6)	1.3 ^b (3.1)
PO ₄ (μM)	1.5 ^a	1.0 ^a (0.8)	1.4 ^a (1.0)	1.1 ^a (0.9)	1.8 ^a (1.5)
NH ₄ (μM)	1.0 ^a	1.3 ^a (0.5)	1.5 ^a (0.9)	1.4 ^a (0.5)	1.9 ^a (1.8)
Sediment H ₂ S (mg/l)	—	0.3 ^a (0.1)	0.3 ^a (0.1)	0.4 ^a (0.1)	0.2 ^a (0.1)
Water ATP (μg/l)	0 ^a	2.8 ^b (1.1)	3.5 ^{bc} (1.2)	3.9 ^{cd} (1.7)	4.5 ^d (2.1)
Sediment ATP (ng/g-dry)	—	0.4 ^a (0.4)	0.3 ^a (0.3)	0.7 ^a (0.6)	0.5 ^a (0.5)
Chl-a (μg/l)	0 ^a	7.6 ^b (4.3)	6.5 ^b (3.3)	8.1 ^b (4.6)	8.4 ^b (2.9)
Chl-a/wATP	—	2.7 ^a	1.8 ^a	2.1 ^a	1.9 ^a

^{a,b,c,d}Mean values across rows with different superscripts were significantly different ($P < 0.05$).

While the two diatoms dominated phytoplankton populations, periodic blooms of several other algae were observed. During the first few weeks after filling, all four ponds sampled had *Chlorella* blooms of up to 20 000 cells/ml. By week 5, *Chlorella* disappeared from the ponds and was not seen again. During week 4, the blue-green algae, *Coccochloris* sp., bloomed dramatically in all four ponds and disappeared by week 5. The dinoflagellate, *Gymnodinium*, bloomed in three of the ponds in week 5, and in pond 6 in week 6, and then disappeared. The primitive green flagellate, *Heteromastix*, bloomed in three of the ponds during week 1, when shrimp were first stocked, and then disappeared. Ciliates and rotifers were found in zooplankton samples from all ponds. Copepods and other crustacean zooplankters were not observed in any of the samples. The attached diatoms, *Navicula* sp. and *Pleurosigma* sp., were the major benthic flora found in the ponds. Nematodes, amoebae and ciliates were the dominant benthic fauna.

Shrimp survival averaged $70.8 \pm 6.3\%$ and there were no significant differences among densities or ponds (Table 4). Shrimp production ranged from 12.3 to 23.0 kg ha⁻¹ day⁻¹ and was significantly higher ($P < 0.05$) in the 10, 15, and 20 shrimp/m² treatments than in the 5/m² ponds. At week 9, shrimp

TABLE 3

Phytoplankton observed in four ponds over the 11-week experimental growout period, ranked by relative abundance and relative frequency

Phytoplankton	Rel. abund. (% of total)	Rank	Rel. freq. (% of total)	Rank
<i>Chaetoceros</i> (D)	38.9	1	23.0	1
<i>Rhizosolenia</i> (D)	34.7	2	20.5	2
<i>Cymbella</i> (D)	10.2	3	0.6	15
<i>Coccolithis</i> (BG)	8.1	4	7.5	6
<i>Peridinium</i> (Dino)	3.3	5	5.0	8
<i>Heteromastix</i> (FL)	1.3	6	3.7	9
<i>Ochromonas</i> (GF)	1.3	7	8.7	3
<i>Chlorella</i> (G)	0.9	8	5.6	7
<i>Gymnodinium</i> (Dino)	0.5	9	8.1	5
<i>Cryptomonas</i> (C)	0.3	10	8.1	4
<i>Navicula</i> (D)	0.2	11	0.6	14
<i>Microcoleus</i> (BG)	—	—	3.7	10
<i>Gyrodinium</i> (Dino)	—	—	1.9	11
<i>Pyramimonas</i> (FL)	—	—	1.2	12
<i>Isochrysis</i> (GF)	—	—	1.2	13
<i>Nitzschia</i> (D)	—	—	0.6	16

Taxa: D=diatoms; BG=bluegreens; Dino=dinoflagellates; G=green; C=cryptomonads; GF=golden flagellates; FL=green flagellates.

in ponds at 5 and 10/m² had grown more rapidly and were significantly larger ($P < 0.05$) than shrimp from ponds at 15/m² which were significantly larger ($P < 0.05$) than shrimp from the ponds with 20 shrimp/m². The estimates of carrying capacity at densities of 10, 15, and 20 shrimp/m² are not significantly different while 5 shrimp/m² was significantly lower (Table 5). A plot of shrimp growth by stocking density is presented in Fig. 1.

DISCUSSION

The higher average pH values in the afternoon, relative to morning, resulted from photosynthetic activities of the phytoplankton populations. Another direct result of photosynthetic activity is oxygen production, demonstrated by the significantly higher DO values in the afternoon compared to the morning. It is unknown if large diurnal DO fluctuations affect shrimp growth. It is generally held that the lower DO limit for shrimp growth is 2 p.p.m. Morning DO levels fell below 2 p.p.m. only once during the course of the experiment.

Incoming seawater to the ponds had significantly higher levels of dissolved nitrate plus nitrite nitrogen than water in the ponds despite weekly additions of nutrients via cattle manure. These dissolved nutrients were rapidly removed from solution in the ponds.

TABLE 4

Means and standard deviation (in parentheses) of individual body weights, growth, biomass increase, and production through 9 weeks and survival at harvest after the 11-week growout period

Stocking density (no./m ²)	5	10	15	20
Initial weight (g)	2.7	2.7	2.7	2.7
Individual body weight	18.1 ^a (1.4)	17.1 ^a (0.7)	12.4 ^a (0.2)	8.7 ^c (0.04)
Growth (g/week)	1.72 ^a (0.2)	1.6 ^a (0.1)	1.1 ^b (0.02)	0.68 ^c (0.00)
Survival (%)	69 ^a (15)	72 ^a (10)	83 ^a *	79 ^a (1)
Biomass increase (g/m ²)	77.2 ^a (7.0)	143.5 ^b (7.4)	145.1 ^b (2.4)	121.6 ^c (1.0)
Production (kg ha ⁻¹ day ⁻¹)	12.3 ^a (1.1)	22.8 ^b (1.2)	23.0 ^b (0.4)	19.3 ^b (0.1)

^{a,b,c}Mean values across rows with different superscripts were significantly different ($P < 0.05$).

*No replicate at harvest.

There was no apparent relationship between water nutrient levels and chlorophyll among ponds and stocking densities throughout the trial period. Shrimp growth was not correlated with any of these data. Rubright et al. (1981) also found no difference in water nutrients among the treatments of feeding, fertilized and no feed.

The absence of coliform bacteria in the pond water combined with no *Salmonella* sp. detected in any shrimp suggests that using manure in shrimp ponds presents little risk of pathogen contamination of harvested shrimp. The public health issues of using manure in aquacultural systems are complex. The legality of this practice for a commercial farm is still uncertain. It is urgent that these legal issues be addressed in the near future.

TABLE 5

An estimation of manure-fed shrimp pond carrying capacity at four stocking densities

Stocking density (shrimp/m ²)	Body weight of zero growth (g)	Carrying capacity (kg/ha)
5	19.0	950 ^a
10	17.0	1700 ^b
15	12.4	1860 ^b
20	8.75	1750 ^b

^{a,b}Column means with different superscripts were significantly different ($P < 0.05$).

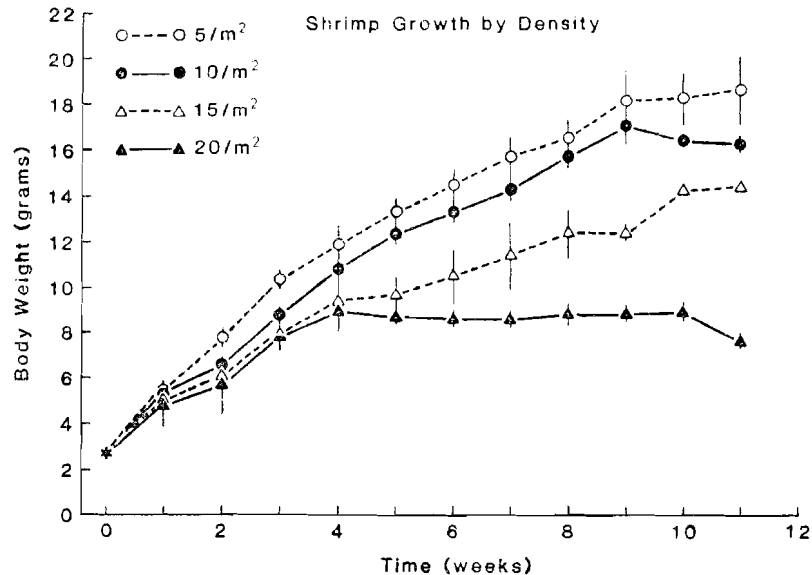


Fig. 1. Shrimp growth at four densities measured weekly in eight ponds during 11-week experimental growout. Points are mean values (\pm SE) for two replicates except for 15/m²; $N=1$ after week 9.

There was a significant ($P < 0.01$) difference in water ATP among stocking densities using Friedman's method (Sokal and Rohlf, 1969). ATP measurements are considered a measure of total microbial biomass (Karl and Craven, 1980) and chlorophyll-a is a measure of autotrophic biomass. The differences among treatments for ATP were probably the result of differences in nonautotrophic (e.g., heterotrophic) biomass.

Chl-a/wATP ratios can be used to estimate the relative amounts of autotrophic and heterotrophic biomass in an aquatic ecosystem. Typical Chl-a/wATP ratios for pure phytoplankton cultures are usually in the range 10-15 (Laws et al., 1984). In this study, Chl-a/wATP ranged from 1.8 to 2.7, suggesting a large contribution to total biomass of nonautotrophic organisms such as bacteria, ciliates, protozoans, etc. It has been proposed that manure-driven, freshwater ecosystems have elevated levels of heterotrophic productivity (Schroeder, 1978; Costa-Pierce et al., 1985). Our results support the extension of that proposal to seawater shrimp ponds.

The dominance and relative stability of the two diatom blooms, *Chaetoceros* and *Rhizosolenia*, are noteworthy. Benthic flora were also dominated by the attached diatoms, *Navicula* and *Pleurosigma*. Generally, the presence of diatoms in shrimp ponds is a desirable condition (Phillips, 1984; Shigueno, 1985). The contribution of diatoms to shrimp nutrition may stem from their high

concentrations of polyunsaturated fatty acids (Orcutt and Patterson, 1974) which are known to promote growth in crustaceans (Kanazawa et al., 1979).

Blooms of other phytoplankton species were coincident among ponds. These transient blooms probably resulted from climatic events such as high winds or changing wind patterns that either stirred up the ponds, resulting in conditions favoring the transient species, or blew in new inoculum that bloomed and later failed.

Trimble (1980) reported that *P. vannamei* grew 1.2 g/week at a stocking density of 2.5 shrimp/m². Our study found growth of 1.72 g/week at a stocking density of 5 shrimp/m². In coastal lagoons in Mexico with an abundance of wild prey organisms and shrimp at densities of 2-3 shrimp/m², *P. vannamei* grew 1.42 g/week (Menz and Blake, 1980). In ponds stocked at 8.5 shrimp/m², ponds receiving both feed and chemical fertilizer had the highest growth rates of several feeding schemes tested (Rubright et al., 1981). It is clear that cattle-manure fertilization of marine shrimp ponds stimulates a food web in the pond that allows outstanding growth rates for *P. vannamei*.

Average individual growth rates were negatively correlated with stocking density ($r^2 = -0.98$; $P < 0.01$); growth rates at the highest stocking density were approximately 40% of those at the lowest stocking density. These results illustrate the complex relationships between feed availability, growth rates, and production. Apparently, the manuring rates used in this study stimulate the development of a certain level of natural food suitable for shrimp resulting in a carrying capacity of about 1700 kg/ha. When densities are increased, individual growth decreases as a result of increased competition for food. If densities are decreased, individual growth increases in response to the decrease in competition for food. In order to increase densities and sustain or increase growth rates, carrying capacity must be increased. We hypothesize this can be accomplished through supplemental feeding and work is in progress to further define these parameters.

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