

# Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*

Wilson Wasielesky Jr.<sup>a,\*</sup>, Heidi Atwood<sup>b</sup>, Al Stokes<sup>b</sup>, Craig L. Browdy<sup>b</sup>

<sup>a</sup> *Laboratório de Maricultura, Departamento de Oceanografia, Fundação Universidade Federal do Rio Grande (FURG), C.P. 474, Rio Grande (RS), 96201-900, Brazil*

<sup>b</sup> *Waddell Mariculture Center – SC, Department of Natural Resources P.O. Box 809, Bluffton, South Carolina 29910, USA*

Received 9 November 2005; received in revised form 17 March 2006; accepted 4 April 2006

## Abstract

Zero water exchange, super-intensive culture of shrimp in enclosed raceway type systems can be considered environmentally friendly in that containment of water within the system prevents potential spread of disease between the wild populations and cultured animals and avoids nutrient rich waste from polluting coastal waters. However, as a relatively new strategy for shrimp production, there is much still to be learned about the potential biological and economic benefits of producing shrimp in suspended microbial floc based systems. Understanding shrimp feeding behavior and quantification of shrimp feed consumption provides valuable information for culturists to improve feed management, one of the keys to economic viability. The objective of this study was to evaluate the nutritional contribution of varying levels of microalgae/bacterial floc on survival, growth, food consumption, and FCR of *Litopenaeus vannamei* juveniles fed diets with different protein levels in replicated experimental microcosm tanks. The 20 day experiment evaluated 9 treatments, three water types fed three different protein diets. Water was recirculated within a sump and consisted of either clear, UV filtered water, water containing microbial floc from an adjacent zero exchange super-intensive raceway production unit, or a 50:50 mix of clear water and raceway water. Diet treatments were either no food, 25% or 35% protein content. Treatments were randomly assigned to 50 L, mesh covered plastic bins receiving each water type. Each treatment consisted of five replicates, each containing 44 shrimp, with a mean stocking weight of  $1.82 \pm 0.71$  g for a final density of 300 per  $m^2$ . Shrimp in each treatment (except the no feed treatment) were fed 3 times daily via a specially designed feed tray. Food consumption and FCR were calculated based on weight gain, survival, total consumed feed, feed loss through leaching, and initial feed moisture content. Results were analyzed by two-way analysis of variance (ANOVA) and differences between the means analyzed by Tukey's test ( $\alpha=0.05$ ). Survival in the fed treatments was greater than 98% in all treatments ( $P>0.05$ ). Survival in the non-fed treatments was significantly higher in the raceway water treatments than in the clear water treatment ( $P<0.05$ ). Final weight, weight gain, final biomass, food consumption and FCR were significantly higher ( $P<0.05$ ) in all treatments fed with 35% protein feed. This result suggests a positive relationship between the growth parameters and the protein content of the feeds in this system, and confirms the benefit of natural productivity for production of *L. vannamei*.

© 2006 Elsevier B.V. All rights reserved.

**Keywords:** Natural production; Microbial floc; *Litopenaeus vannamei*; Greenhouse system

\* Corresponding author. Tel.: +55 53 3236 8131; fax+55 53 3236 8042.  
E-mail address: [manow@mikrus.com.br](mailto:manow@mikrus.com.br) (W. Wasielesky).

## 1. Introduction

Shrimp production methods in the western hemisphere have evolved based on three production strategies. During the 1980's shrimp production was based primarily on high surface area, low-density pond production. For example in Ecuador production reached 100,000 tons/year with an average productivity of roughly 500 kg/ha/year. Subsequently, strategies shifted to incorporate improved technologies including fertilization, use of feeding trays, and increased stocking densities.

During the 1990's, strategies were demonstrated in the United States for pond production of up to 5000 kg/ha of *Litopenaeus vannamei* with little or no water exchange (Hopkins et al., 1995; Browdy et al., 2001). The advantages of these techniques included reduced water use, reduced wastewater discharge and less environmental impact due to nutrient discharge during the production cycle. According to Cuzon et al. (2004), in 1998 western hemisphere production was 132,000 tons. Concurrent with the incorporation of the low or zero exchange strategies, production increased to 271,000 tons in 2003 with the greatest increase in Brazil where production reached 90,190 tons with average productivity of up to 6000 kg/ha/year (Rocha et al., 2004). Despite the increase in production over this time period, diseases became problematic, especially the Taura Syndrome Virus which severely impacted production in several South American shrimp farms (Brock et al., 1997), the White Spot Syndrome Virus causing mortalities in the Americas (Lightner, 1999), and more recently mortalities attributed to the Infectious Monocytosis Virus in northeast Brazil (Lightner and Pantoja, 2004; Nunes et al., 2004). Zero exchange pond production reduced the risk of introduction and spread of disease while providing the nutritional benefits of natural productivity within ponds (McIntosh et al., 2000; Bratvold and Browdy, 2001; Moss et al., 2001; Samocha et al., 2001; Weirich et al., 2002; Burford et al., 2003). Some of the highest production levels have been achieved in very low exchange systems based on suspended microbial floc communities. Browdy et al. (2001) reported an average production of 15 tons/ha in these types of ponds in Belize. Although high levels of productivity can be achieved in open ponds, production is limited to one or two crops/year in most temperate to tropical locations (Kumlu et al., 2001; Tidwell et al., 2003) and biosecurity remains an important concern (Weirich et al., 2003).

During the last few years researchers, have been developing newer production methods which move

shrimp production indoors, into greenhouse enclosed raceway systems (Browdy and Moss, 2005). Like the recent improvements in pond production, covered raceways operate with zero water exchange and benefit from the enhanced natural productivity that this strategy provides. Additionally, covered raceway systems offer an increased level of biosecurity, being an essentially closed system, and a capability of producing shrimp year-round. In South Carolina, at the Waddell Mariculture Center, greenhouse enclosed raceway systems have been in use since 2000 and production has increased from 3 kg/m<sup>2</sup> per crop (McAbee et al., 2003) to the most recent production of 6.8 kg/m<sup>2</sup> (McAbee, personal communication). Shrimp densities in these systems have increased from 300 to 500/m<sup>2</sup> with survival rates of >70% and growth rates in excess of 1.5 g/week in the suspended microbial floc raceway environment.

It has been suggested that the enhanced natural productivity in zero exchange shrimp production systems allows for the use of lower protein feeds. Use of lower protein feeds is more cost effective and more environmentally friendly because the fishmeal component is reduced. It is known that natural production can supplement shrimp feeding as observed by Moss (2002) and Decamp et al. (2002). Browdy et al. (2001) also showed the positive effect of natural productivity achieving similar production of *L. vannamei* in ponds fed on 30% and 45% crude protein (CP). Samocha et al. (2004), however, reported no statistical differences between the growth parameters of *L. vannamei* fed on 30% and 40% protein, in intensively managed tanks without water exchange. Replicated microcosm systems fed with water from intensively managed zero exchange raceways can provide a powerful experimental system for the evaluation of feeds at high densities under heterotrophic raceway conditions providing valuable data for development of feed management strategies for these types of systems.

The objective of this study was to evaluate the nutritive value of varying concentrations of suspended microalgal/bacterial floc on survival, growth, food consumption, and FCR of *L. vannamei* juveniles fed diets containing varying amounts of protein.

## 2. Materials and methods

This study was conducted from May until August 2004 in a small greenhouse at the Waddell Mariculture Center (WMC), South Carolina Department of Natural Resources, Bluffton, South Carolina. *L. vannamei*

post-larvae were obtained from Shrimp Improvement Systems (SIS), Islamorada, Florida, and were maintained in a greenhouse nursery system for two months prior to use. The nursery system was a high density polyethylene (HDPE) lined, sediment-free, greenhouse enclosed raceway system (55 m<sup>2</sup>) equipped with AquaMats™ attached to free-standing PVC frames used for vertical surface enhancement and nitrification. Aeration and circulation for the raceway was provided by blown air. Shrimp were stocked at a density of 230/m<sup>2</sup> and fed the same 35% CP diet used during the study. Juveniles used in the study were netted and transferred to the microcosm experimental units and allowed to acclimate for at least one day prior to starting the study.

The experimental microcosm system consisted of 45 plastic bins (50 L) each with a bottom area of 0.148 m<sup>2</sup>. There were 15 bins for each of the 3 water treatments (raceway water, clear water, and a 50:50 mix of raceway and clear water). All clear water used for this experiment was pumped from Colleton River, filtered and UV sterilized. Each set of bins had a common 3000 L fiberglass reservoir. The bins from each water treatment received water recirculated from the corresponding reservoir at a rate of 50 exchanges/day. Water in each reservoir was renewed daily. Each bin was stocked with 44 juvenile shrimp, mean weight 1.82±0.71 g for a final stocking density of 300/m<sup>2</sup>. Bins were covered with nylon mesh to prevent escape and bins from each water treatment were randomly assigned to each of the 3 feed treatments: (1) no feed, (2) 25% CP feed, and (3) 35% CP feed. It is important to note that the nominal percent crude protein was based on the commercial label. There were five replicates of each feeding regime per water treatment. Feeds were supplied 3 times/day (0900, 1500, and 2100 h) via a specially designed feeding tray. The feeding tray

design consisted of a ¾" PVC pipe, notched at the bottom, attached by a bolt to the inside edge of a 4 inch. diameter, 1 inch. deep PVC ring with an attached bottom made from 105 µm mesh. The design of the tray assured distribution of rations over the entire tray surface. Uneaten feed was removed from the feed trays every morning, dried in an oven at 70 °C until a constant weight was achieved and the final dry weight was then recorded to the nearest 0.01 g. Feed leaching and percent moisture were measured to determine feed stability prior to starting the experiment. Feed consumption and FCR were determined based on weight gain, survival, total feed consumed, feed lost through leaching and initial feed moisture content. Temperature, salinity, pH, transparency (Secchi), and dissolved oxygen were measured daily and TA-N, NO<sub>2</sub>-N, NO<sub>3</sub>, and chlorophyll α were measured twice weekly. At the end of the experiment, shrimp from each treatment were pooled for analysis of proximate composition. Raceway water was filtered through a 5 µm filter bag to collect suspended material (bacterial floc, microalgae, and sludge) present in inlet water. Shrimp, collected solids, and feed samples were frozen and shipped to Eurofins Bioanalytical Company, Memphis, Tennessee, USA, for analysis.

Survival and final wet weight were determined and recorded for each of the 45 bins at the end of the 20 day study and results were analyzed by two-way ANOVA with treatment means compared by Tukey's HSD test ( $\alpha=0.05$ ).

### 3. Results

There was no significant difference for any measured water quality parameter within each water treatment. There were, however, significant differences among

Table 1  
Water-quality parameters from each treatment reservoir

Parameter	Reservoir 1	Reservoir 2	Reservoir 3	P
	Greenhouse water	Mixed water	Clear water	
Temperature (C)	27.57(1.31) <sup>A</sup>	27.96(1.30) <sup>A</sup>	28.06(1.49) <sup>A</sup>	0.3231
Salinity (g/L)	31.61(0.71) <sup>A</sup>	31.86(0.43) <sup>A</sup>	32.20(0.45) <sup>B</sup>	0.0004
pH	7.65(0.18) <sup>A</sup>	7.72(0.16) <sup>AB</sup>	7.81(0.14) <sup>B</sup>	0.0087
Dissolved oxygen (mg/L)	6.16(0.79) <sup>A</sup>	6.26(0.79) <sup>A</sup>	6.52(0.89) <sup>A</sup>	0.2294
Secchi (cm)	30.99(7.86) <sup>A</sup>	43.98(8.35) <sup>B</sup>	Total	0.0000
Chlorophyll α (µg/L)	103.80(50.46) <sup>A</sup>	61.66(27.04) <sup>B</sup>	9.63(6.97) <sup>C</sup>	0.0000
TA-N (mg/L)	0.47(0.63) <sup>A</sup>	0.17(0.21) <sup>AB</sup>	0.13(0.14) <sup>C</sup>	0.0000
NO <sub>2</sub> -N (mg/L)	0.68(0.16) <sup>A</sup>	0.38(0.10) <sup>B</sup>	0.06(0.05) <sup>C</sup>	0.0000
NO <sub>3</sub> -N (mg/L)	3.81(1.21) <sup>A</sup>	2.48(1.31) <sup>B</sup>	1.10(0.76) <sup>C</sup>	0.0000

Data are means, standard error, and significance level, along experiment. Same letter indicate (in column) means are not significantly different ( $P>0.05$ ).

Table 2

Proximate composition of 25% and 35% crude protein diets as indicated on the commercial label (CL) and by laboratory analysis (LA), and dry suspended particulate matter (microbial floc) present in greenhouse water

	25% CL	25% LA	35% CL	35% LA	Floc
Protein crude (%)	25 (min)	29.05	35 (min)	40.55	31.07
Fat/oil (%)	9 (min)	9.14	9 (min)	8.70	0.49
Carbohydrate (%)	45 (max)	42.74	35 (max)	28.51	23.59
Ash (%)	9 (max)	8.2	9 (max)	8.78	44.85
Moisture (%)	10 (max)	10.87	10 (max)	9.46	–
Calories (kcal/kg)	2600.00	3690.00	2900.00	3710.00	2233.01
ME (kcal/kg)	2300.00	3247.35	2500.00	3192.24	2011.95

\*min — minimum percent must include in feed. \*\*max — maximum percent must include in feed.

treatments (Table 1) for salinity, Secchi, Chlorophyll  $\alpha$ , and inorganic nitrogen parameters ( $P < 0.05$ ).

Proximal analysis of feed indicated that actual crude protein content was higher in the feed than was indicated on the label. The caloric content and metabolizable energy (ME) of the feeds were similar (Table 2). The composition of suspended particulate matter (“microbial floc”) in the raceway water had high levels of ash and protein (Table 2).

There were no significant differences ( $P > 0.05$ ) in survival between the water treatments for shrimp fed 25% or 35% CP diets (range = 98.2% to 100%) but there was a significant difference between water treatments for unfed shrimp ( $P < 0.05$ ) (Table 3). Mean growth rate differed between feed treatments and was significantly different for each water treatment ( $P < 0.05$ ). There was

no significant difference in growth between the water treatments for either the no feed or 25% CP diet treatments. There was, however, a significant difference between water treatments for shrimp fed the 35% CP diet with better growth observed in the raceway water treatments ( $P < 0.05$ ) (Fig. 1).

Mean final weight, mean weight gain, mean final biomass, and biomass/m<sup>2</sup> were significantly higher ( $P < 0.05$ ) in 35% CP diet raceway water treatments. There was no significant difference in mean final weight, mean weight gain, mean final biomass, and biomass/m<sup>2</sup> in raceway water treatments for shrimp fed the 25% CP diet. Unfed shrimp showed reduced growth but were not significantly smaller in the clear water treatment than the raceway water treatments ( $P > 0.05$ ). Food consumption was significantly higher ( $P < 0.05$ ) in the 35% CP than in the 25% CP diet treatments but there was no significant difference between the water treatments ( $P > 0.05$ ) for shrimp fed either the 35% CP or 25% CP diets (Table 3).

In spite of better growth in the 35% CP diet treatments (as measured by the mean final weight, mean final biomass, mean biomass gain, and mean biomass/m<sup>2</sup>), there was no statistical difference in FCR for mixed greenhouse water or greenhouse water alone (range 1.03:1 to 1.21:1). Fig. 2 shows that FCR in clear water treatments were significantly higher ( $P < 0.05$ ).

Proximate analysis of shrimp tissue fed either the 25% or 35% crude protein diets showed higher levels of protein and fat than did tissue from unfed shrimp. Percent protein in fed shrimp ranged from 16.17% to 18.51% and from 10.56% to 11.53% in unfed

Table 3

Growth parameters of Pacific white shrimp *L. vannamei* in different water environments and different feeding regimes

Treatment	Mean survival (%)	Mean final weight (g)	Mean final biomass (g)	Mean biomass gain/loss (g)	Mean biomass/m <sup>2</sup> (g)	Mean food consumption per replica
<i>Greenhouse water</i>						
No food	76.81 <sup>B</sup> (2.49)	1.46 <sup>A</sup> (0.09)	49.34 <sup>B</sup> (3.00)	30.65 <sup>*B</sup> (5.34)	337.96 <sup>B</sup> (20.56)	–
25% CP	100.00 <sup>C</sup> (0.00)	3.19 <sup>B</sup> (0.13)	140.56 <sup>C</sup> (5.77)	61.62 <sup>C</sup> (2.88)	962.74 <sup>C</sup> (39.49)	65.31 <sup>A</sup> (1.11)
35% CP	99.09 <sup>C</sup> (5.43)	5.43 <sup>D</sup> (0.36)	236.78 <sup>E</sup> (13.58)	155.21 <sup>E</sup> (16.21)	1621.80 <sup>E</sup> (93.05)	158.81 <sup>B</sup> (6.10)
<i>Mixed water</i>						
No food	65.91 <sup>B</sup> (6.43)	1.48 <sup>A</sup> (0.10)	42.72A <sup>B</sup> (1.88)	37.63 <sup>*B</sup> (7.03)	292.58 <sup>AB</sup> (12.86)	–
25% CP	100.00 <sup>C</sup> (0.00)	3.08 <sup>B</sup> (0.12)	135.42 <sup>C</sup> (5.38)	56.40 <sup>C</sup> (8.75)	927.56 <sup>C</sup> (36.83)	67.02 <sup>A</sup> (3.00)
35% CP	99.09 <sup>C</sup> (1.24)	5.39 <sup>D</sup> (0.39)	234.82 <sup>E</sup> (15.03)	153.34 <sup>E</sup> (16.85)	1608.38 <sup>E</sup> (102.97)	156.68 <sup>B</sup> (7.07)
<i>Clear water</i>						
No food	38.18 <sup>A</sup> (9.83)	1.66 <sup>A</sup> (0.23)	27.57 <sup>A</sup> (6.23)	53.04 <sup>*A</sup> (8.58)	188.84 <sup>A</sup> (42.72)	–
25% CP	98.18 <sup>C</sup> (2.96)	2.92 <sup>B</sup> (0.35)	126.46 <sup>C</sup> (17.69)	47.33 <sup>C</sup> (18.40)	886.01 <sup>C</sup> (121.14)	65.96 <sup>A</sup> (10.52)
35% CP	99.09 <sup>C</sup> (1.24)	4.29 <sup>C</sup> (0.26)	186.85 <sup>D</sup> (11.32)	105.71 <sup>D</sup> (10.94)	1279.77 <sup>D</sup> (77.51)	145.89 <sup>B</sup> (8.69)
<i>P</i>	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Data are means, standard error, and significance level. Same letter indicate (in column) means are not significantly different ( $P > 0.05$ ).

\* Decrease of biomass.

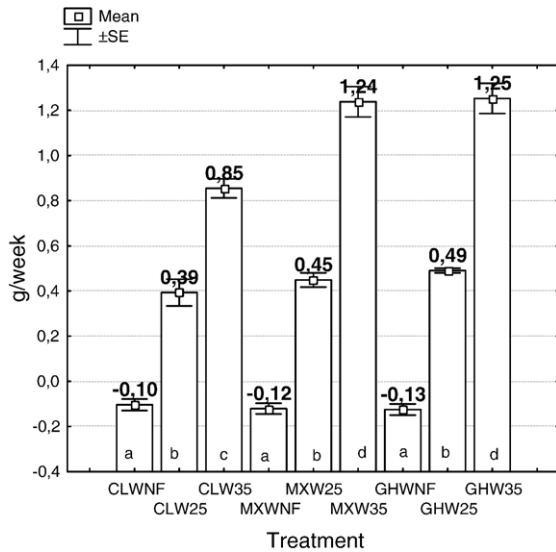


Fig. 1. Mean growth rate (g/week) of Pacific white shrimp *L. vannamei* in different environments: clear water (CLW), mixed water (MXW) and greenhouse water (GHW), and different feedings regimes: no food added (NF), 25% CP, and 35% CP diets. Same letters indicate means that are not significantly different ( $P > 0.05$ ).

animals Percent crude fat/oil ranged from 1.08% to 2.12% in fed shrimp and from 0.49% to 0.52% in unfed animals. On the other hand, the moisture content was higher in unfed shrimp. Percent moisture in fed shrimp ranged from 76.31% to 78.46%, and to from 84.24% to 85.15% (Table 4) in unfed animals.

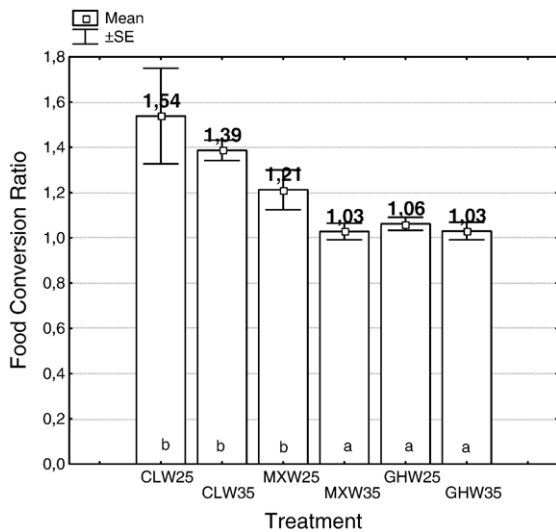


Fig. 2. Food conversion rate for Pacific white shrimp *L. vannamei* reared in different environments: clear water (CLW), mixed water (MXW) and greenhouse water (GHW), and different feeding regimes: 25% CP, and 35% CP diets. Same letters indicate means that are not significantly different ( $P > 0.05$ ).

#### 4. Discussion

There were significant differences between water treatments for some water quality parameters. Salinity was slightly higher in the clear water reservoir, probably due to high evaporation, in the river that was the salt water source, during summer months. The pH was lower in the raceway- and mixed water treatment reservoirs, probably the result of respiration by heterotrophic organisms, which increased the carbon dioxide concentration in the greenhouse system from which the raceway water was pumped. Concentration of inorganic nitrogen by-products was significantly higher in raceway water treatments due to increased metabolism associated with the rich microbial community in these bins. Mean temperature and dissolved oxygen were not significantly different between the water treatments. Despite differences in water quality parameters among treatments, all water quality parameters were within acceptable ranges for survival and growth for *L. vannamei* (Van Wyk and Scarpa, 1999; Wickins, 1976). Light penetration and productivity as measured by Secchi and chlorophyll  $\alpha$  were consistent with those observed during low water exchange suspended floc based shrimp culture in raceways (Weirich et al., 2003) and in ponds (Burford et al., 2004).

Because *L. vannamei* can be cultured under a variety of environmental conditions at high densities it is the species of choice for super-intensive production systems (Cuzon et al., 2004). Williams et al. (1996) evaluated the effect of stocking density on survival of *L. vannamei* with survival ranging from 95.0% to 81.3%, at stocking densities ranging from 28.4 to 284/m<sup>2</sup>. During this study *L. vannamei* were reared at a density of 300/m<sup>2</sup>, with survival in fed treatments in excess of 98%, demonstrating the high tolerance of this species to super-

Table 4

Proximate composition of Pacific white shrimp *L. vannamei* (whole body) reared in different environments: greenhouse water (GHW), mixed water (MXW) and clear water (CLW), and different feeding regimes: with no food added (NF), 25% CP, and 35% CP diets

	Protein (%)	Fat/oil (%)	Carbohydrate (%)	Ash (%)	Moisture (%)
GHW NF	11.53	0.49	0.35	3.39	84.24
GHW 25	17.24	1.12	0.83	3.24	77.57
GHW 35	18.51	1.86	0.34	2.98	76.31
MXW NF	10.58	0.50	0.83	3.40	84.69
MXW 25	16.50	1.08	0.96	3.22	78.24
MXW 35	18.22	2.12	0.28	3.01	76.37
CLW NF	10.56	0.52	0.50	3.27	85.15
CLW 25	16.17	1.28	1.00	3.12	78.46
CLW 35	18.15	1.96	0.50	3.02	76.37



intensive rearing conditions and the efficacy of the experimental system. Survival of shrimp in the unfed treatments was significantly lower (76.81% in the raceway water treatment, 65.91% in the mixed water treatment, and only 38.18% in the clear water treatment) reflecting the contribution of natural production to survivability for starved shrimp. The difference in survival confirms the benefit that heterotrophic bacteria, phytoplankton and other microorganisms present in raceway water can have for survival and growth of juvenile *L. vannamei*. Leber and Pruder (1988) observed that shrimp derive nutrition from natural productivity, and can have high survival even without feed addition at semi-intensive stocking densities. Burford et al. (2004) reported that up to 29% of daily feed intake of *L. vannamei* could be bacterial microalgae/bacterial floc from the heterotrophic culture system. Similar results were observed by Moss et al. (1992) by analyzing growth of *L. vannamei* while removing different solid fractions from the culture water. The authors confirmed the consumption of microalgae and microbial-detrital aggregates from the culture water. These microbial components contribute, to some extent, to the survival and growth of unfed shrimp in a heterotrophic culture system depending on stocking density, shrimp biomass, shrimp size, water quality. Current experimental results indicate that the microbial community is a significant nutrient source for raceway and mixed water treatment animals, supporting a live mean biomass equivalent to 337.97 and 234.82 g/m<sup>2</sup>, respectively.

No significant effect of culture water was found for growth among 25% CP diet treatments. This may have been related to the overall slow growth rate for this diet resulting in insufficient growth to determine the effect of natural productivity. McIntosh et al. (2000) did not detect significant differences, after 94 days, in survival and mean final weight in *L. vannamei* fed 21% protein feed, with a bacterial supplement. Results of the current experiment suggest that the reduction in growth associated with the feeding of a low protein diet in the super-intensive system (300/m<sup>2</sup>) was not reduced by natural productivity (microalgal/bacterial floc). Natural productivity can supplement a higher protein diet as demonstrated with the 35% crude protein diet where *L. vannamei* obtained better growth in raceway and mixed water treatments and biomass gains were at least 45% higher where natural productivity was available. This result corroborates studies conducted by Leber and Pruder (1988), Otoshi et al. (2001), Decamp et al. (2002) where better growth was obtained in *L. vannamei* fed a high protein diet in water with natural productivity. The better growth observed in the 35% CP diet treatment in

this study, is directly related to the important nutritive value of microorganisms present in the raceway water source (Moss et al., 2001). According to Jory (2001) and Tacon et al. (2002) bacterial floc contains high levels of protein and other important factors that supplement shrimp nutrition. Proximal analysis of bacterial floc and microalgae components of the microbial community from the current experiment indicates the presence of a high protein level. The microalgal/bacterial floc protein level was comparable to 31.2% CP detected by Tacon et al. (2002) and less than 43% CP reported by Jory (2001). The percent ash observed in the current study was higher, by comparison, than observed in these other studies. This may be related to the higher amount of shrimp fecal matter in suspension in the raceway water (stocked at 230/m<sup>2</sup>) used and in the experimental bins (stocked at 300/m<sup>2</sup>). In contrast, the fat/oil content of the bacterial floc was significantly lower than that cited by Jory (2001) and Tacon et al. (2002). At 31.07% protein (dry based), the suspended material in the raceway water successfully augmented dietary protein. High survival and growth rates up to 1.20 g/week were similar to survival and growth rates obtained by McAbee et al. (2003) and Weirich et al. (2002) who achieved growth rates above 1.44 and 1.30 g/week, respectively, in raceways. The results obtained with the 35% CP diet also confirm that even at high stocking densities, natural production plays an important role in target crop nutrition.

Another important point is the comparison between lower and higher protein feeds for shrimp culture. Recently, several works have explored rearing *L. vannamei* using low protein levels feeds, in high primary and secondary production water, with no or lower exchange water rates. Samochoa et al. (2004) presented the results of several experiments that showed no significant difference in survival and growth of shrimp fed with 30% and 40% CP; 39% and 43% CP, and 25% and 35% CP diets fed on an isonitrogenous basis. Likewise, Browdy et al. (2001) found no significant differences in growth between shrimp fed 30% and 45% CP diets. In contrast, McIntosh et al. (2001) found significant differences in growth parameters of *L. vannamei* fed 21% and 31% CP diets. Venero et al. (2005) observed the same differences feeding 30% and 40% CP diets to *L. vannamei* in tanks. The results observed in the current study were similar to McIntosh et al. (2001) and Venero et al. (2005), in that shrimp fed the 35% CP diet reached a mean final weight that was 70.2%, 75.0%, and 46.9% higher than the shrimp fed the 25% CP diet, in raceway water, mixed water, and clear

water, respectively, showing again the positive effect of natural production when fed a higher protein diet. According to these results, the growth enhancement effect of the natural productivity was better when a higher protein diet was offered to the shrimp.

Food consumption was significantly different between shrimp fed 25% and 35% CP diets. Feed consumption was over two times higher for shrimp fed the 35% CP diet than shrimp fed the 25% CP diet. In spite of the difference in feed consumption, there was no significant difference between the FCRs for shrimp fed either the 25% CP or 35% CP diets, in different water conditions. Browdy et al. (2001) and Samocha et al. (2004) detected no significant difference in FCR when feeding *L. vannamei* 30% and 45% CP diets and 39% and 43% CP diets, respectively. Conversely, McIntosh et al. (2001) fed 21% and 31% CP diets; Samocha et al. (2004) fed 30% and 40% CP diets, 25% and 35% CP diets; Venero et al. (2005) fed 30% and 40% CP diets, to *L. vannamei* and all researchers had better FCRs when feeding a higher protein diet. In the current study, FCRs were carefully controlled as an integral part of the experimental design. Nevertheless, FCR was significantly improved by the addition of natural productivity decreasing from 1.39:1 in clear water (35% CP diet) to 1.03:1 in raceway and mixed water (35% CP diet).

Proximate analysis of shrimp tissue showed definite differences between fed and unfed treatment shrimp. Shrimp in the unfed treatments had lower protein and fat/oil levels, and a relatively higher percent moisture and ash content. Proximate analysis of shrimp tissue protein ranged from 16.17% to 18.51% in 25% and 35% CP diet treatments. These results were similar to those obtained by Tacon et al. (2002) which ranged from 17.86% to 19.82% CP. In general, however, the proximate composition of shrimp tissue was primarily influenced by the protein composition of supplied feeds, and secondarily influenced by the presence of natural productivity, demonstrating higher available energy for shrimp fed a 35% CP diet, in raceway water.

## 5. Conclusion

In summary, the microcosm system and methodology utilized in the current study is an efficient way to analyze the effect of natural productivity in a heterotrophic super-intensive culture system on survival, growth, weight gain, food consumption and FCR. It is highly recommended that practical diets for culture of penaeid shrimp in zero exchange suspended microbial floc raceways be tested in experimental systems which provide natural productivity and organic material found

in the water column. Growth parameters were improved by feeding a higher protein diet and this improvement was enhanced by factors associated with water column natural productivity. On the other hand, when feeding a lower protein diet which limits growth, effects of natural productivity may be less discernable. Suspended particulate matter present in the super-intensive culture system can significantly improve FCR, reducing the feed costs and inorganic nitrogen waste associated with shrimp production.

## Acknowledgements

The authors would like to acknowledge the US Marine Shrimp Farming Program, USDA and CNPq-Brazil (Process: 200039/04-0) for the financial support in the execution of this work. This is manuscript number 577 from the Marine Resources Division of the South Carolina Department of Natural Resources.

## References

- Bratvold, D., Browdy, C.L., 2001. Effects of sand sediment and vertical surfaces (AquaMats™) on production, water quality, and microbial ecology in an intensive *Litopenaeus vannamei* culture system. *Aquaculture* 195, 81–94.
- Brock, J.A., Gose, R.B., Lightner, D.V., Hasson, K., 1997. Recent developments and an overview of Taura Syndrome of Farmed shrimp in the Americas. In: Flegel, T.W., McRae, I.H. (Eds.), *Diseases in Asian Aquaculture III*. Fish Health Section, Asian Fisheries Society, Manila, Philippines, pp. 275–284. 1997.
- Browdy, C.L., Moss, S.M., 2005. Shrimp Culture In Urban, Super-intensive Closed Systems. In: Costa Pierce, B.A. (Ed.), *Urban Aquaculture*. Blackwell Science, Oxford UK, pp. 173–186.
- Browdy, C.L., Bratvold, D., Stokes, A.D., McIntosh, R.P., 2001. Perspectives on the application of closed shrimp culture systems. In: Browdy, C.L., Jory, D.E. (Eds.), *The New Wave, Proceedings of the Special Session on Sustainable Shrimp Culture, Aquaculture 2001*. The World Aquaculture Society, Baton Rouge, USA, pp. 20–34.
- Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H., Pearson, D.C., 2003. Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. *Aquaculture* 219, 393–411.
- Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H., Pearson, D.C., 2004. The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero-exchange system. *Aquaculture* 232, 525–537.
- Cuzon, G., Lawrence, A., Gaxiola, G., Rosas, C., Guillaume, J., 2004. Nutrition of *Litopenaeus vannamei* reared in tanks or in ponds. *Aquaculture* 235, 513–551.
- Decamp, O., Conquest, L., Forster, I., Tacon, A.G.J., 2002. The nutrition and feeding of marine shrimp within zero-water exchange aquaculture production system: role of Eukaryotic microorganisms. In: Lee, C.S., O'Bryen, P. (Eds.), *Microbial Approaches to Aquatic Nutrition within Environmentally Sound Aquaculture Production Systems*. World Aquaculture Society, Baton Rouge, USA, pp. 79–86.

- Hopkins, J.S., Sandifer, P.A., Browdy, C.L., 1995. Effect of two feed protein levels and feed rate combinations on water quality and production of intensive shrimp ponds operated without water exchange. *J. World Aquac. Soc.* 26, 93–97.
- Jory, D.E., 2001. Feed management practices for a healthy pond environment. In: Browdy, C.L., Jory, D.E. (Eds.), *The New Wave, Proceedings of the Special Session on Sustainable Shrimp Culture, Aquaculture 2001*. The World Aquaculture Society, Baton Rouge, USA, pp. 118–143.
- Kumlu, M., Erolodogun, O.T., Saglamtimur, B., 2001. The effects of salinity and added substrates on growth and survival of *Metapenaeus monoceros* (Decapoda: Penaeidae) post-larvae. *Aquaculture* 196, 177–188.
- Leber, M.K., Pruder, G.D., 1988. Using experimental microcosm in shrimp research: the growth-enhancing effect of shrimp pond water. *J. World Aquac. Soc.* 19, 197–203.
- Lightner, D.V., 1999. The Penaeid shrimp viruses TSV, IHHNV, WSSV, and YHV. Current status in the Americas, available diagnostic methods and management strategies. *J. Appl. Aquac.* 9, 27–52.
- Lightner, D.V., Pantoja, C.R., 2004. Infectious Myonecrosis (IMN): Current status report in the biology of the etiological agent and development of diagnostic methods. In: Rocha, I.P. (Ed.), *Book of abstract of Fenacam, Natal, Brazil*, p. 40.
- McAbee, B.J., Browdy, C.L., Rhodes, R.J., Stokes, A.D., 2003. The use of greenhouse-enclosed raceway systems for the super-intensive production of pacific white shrimp *Litopenaeus vannamei* in the United States. *Global Aquaculture Advocate* 6 (4).
- McIntosh, D., Samocha, T.M., Jones, E.R., Lawrence, A.L., McKee, D.A., Horowitz, S., Horowitz, A., 2000. The effect of a bacterial supplement on the high-density culturing of *Litopenaeus vannamei* with low-protein diet in outdoor tank system and no water exchange. *Aquac. Eng.* 21, 215–227.
- McIntosh, D., Samocha, T.M., Jones, E.R., Lawrence, A.L., Horowitz, S., Horowitz, A., 2001. Effects of two commercially available low-protein diets (21% and 31%) on water sediment quality, and on the production of *Litopenaeus vannamei* in an outdoor tank system with limited water discharge. *Aquac. Eng.* 25, 69–82.
- Moss, S.M., 2002. Dietary importance of microbes and detritus in Penaeid shrimp aquaculture. In: Lee, C.S., O'Bryen, P. (Eds.), *Microbial Approaches to Aquatic Nutrition within Environmentally Sound Aquaculture Production Systems*. World Aquaculture Society, Baton Rouge, USA, pp. 1–18.
- Moss, S.M., Pruder, G.D., Leber, K.M., Wyban, J.A., 1992. The relative enhancement of *Penaeus vannamei* growth by selected fractions of shrimp pond water. *Aquaculture* 101, 229–239.
- Moss, S.M., Arce, S.M., Argue, B.J., Otoshi, C.A., Calderon, F.R.O., Tacon, A.G.J., 2001. Greening of the blue revolution: Efforts toward environmentally responsible shrimp culture. In: Browdy, C.L., Jory, D.E. (Eds.), *The New Wave, Proceedings of the Special Session on Sustainable Shrimp Culture, Aquaculture 2001*. The World Aquaculture Society, Baton Rouge, USA, pp. 1–19.
- Nunes, A.J.P., Martins, P.C.C., Gesteira, T.C.V., 2004. Carcinicultura ameaçada: produtores sofrem com as mortalidades decorrentes do Vírus da Mionecrose Infecçiosa (IMNV). *Panorama da Aqüicultura*, vol. 14(83), pp. 37–51.
- Otoshi, C.A., Montgomery, A.D., Look, A.M., Moss, S.M., 2001. Effects of diet and water source on the nursery production of pacific white shrimp *Litopenaeus vannamei*. *J. World Aquac. Soc.* 32, 243–249.
- Rocha, I.P., Rodrigues, J., Amorin, L., 2004. The Brazilian shrimp culture in 2003. *Brazilian Association of Shrimp Rearing Magazine*, vol. 6(1), pp. 30–36.
- Samocha, T.M., Lawrence, A., Collins, C.R., Emberson, C.R., Harvin, J.L., Van Wyk, P.M., 2001. Development of integrated, environmentally sound, inland shrimp production technologies for *Litopenaeus vannamei*. In: Browdy, C.L., Jory, D.E. (Eds.), *The New Wave, Proceedings of the Special Session on Sustainable Shrimp Culture, Aquaculture 2001*. The World Aquaculture Society, Baton Rouge, USA, pp. 64–75.
- Samocha, T.M., Patnaik, S., Gandy, R.L., 2004. Heterotrophic intensification of pond shrimp production. *Book of abstract of Fifth International Conference on Recirculating Aquaculture*, 22–25 July 2004, Roanoke, Virginia, USA.
- Tacon, A.G.J., Cody, J.J., Conquest, L.D., Divakaran, S., Forster, I. P., Decamp, O.E., 2002. Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed different diets. *Aquac. Nutr.* 8, 121–137.
- Tidwell, J.H., Coyle, S.D., Bright, L.A., Van Arnum, A., Weibel, C., 2003. The effects of size grading and length of nursery period on growth and population structure of freshwater prawns stocked in temperate zone ponds with added substrates. *Aquaculture* 218, 209–218.
- Van Wyk, P., Scarpa, J., 1999. Water Quality and Management. In: Van Wyk, P., et al. (Eds.), *Farming Marine Shrimp in Recirculating Freshwater Systems*. Florida Department of Agriculture and Consumer Services, Tallahassee, pp. 128–138.
- Venero, J.A., Davis, D.A., Rouse, D.B., 2005. Variable feed allowance with constant protein input for Pacific White Shrimp *Litopenaeus vannamei* in tanks using green water. In: *World Aquaculture Society (Ed.), CD-Rom of Abstracts of the Aquaculture America Shrimp growout, nutrition and Disease session. 2005, 17–20 February 2005, New Orleans, Louisiana, USA*.
- Weirich, C.R., Browdy, C.L., Bratvold, D., McAbee, B.J., Stokes, A. D., 2002. Preliminary characterization of a prototype minimal exchange super-intensive shrimp production system. *Proceedings of the IVth International Conference on Recirculating Aquaculture*. Virginia Tech University, Blacksburg, Virginia, USA, pp. 255–270.
- Weirich, C.R., Segars, A., Bruce, J.V., Browdy, C.L., 2003. Development and implementation of biosecurity protocols and procedures at the Waddell Mariculture Center. In: Lee, C.-S., O'Bryen, P.J. (Eds.), *Biosecurity in Aquaculture Production Systems: Exclusion of Pathogens and Other Undesirables*. The World Aquaculture Society, Baton Rouge, LA USA, pp. 139–156.
- Wickins, J.F., 1976. The tolerance of warm-water prawns to recirculated water. *Aquaculture* 9, 19–37.
- Williams, A.S., Davis, D.A., Arnold, C.R., 1996. Density-dependent growth and survival of *Penaeus setiferus* and *Penaeus vannamei* in a semi-closed recirculating system. *J. World Aquac. Soc.* 27, 107–112.