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Research Article

Long term storage and the compensatory growth of white shrimp *Litopenaeus vannamei* in aquaculture ponds

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ABSTRACT. Effects of shrimp confinement in a situation of high density stocking in a long term nursery on their growth performance in grow out ponds. Were analized two nurseries with a density of 2000 shrimp m^{-2} were stocked at two different times. The first nursery (LTN) lasted 144 days, and the SGR of the animals was 3.0% day^{-1}. The second nursery (STN) lasted 18 days and the specific growth rate (SGR) was 19.9% day^{-1}. On the same day, shrimps were transferred to six lined ponds at a density of 20 shrimp m^{-2} where they remained for 101 days. In the first biometry, the SGR in the LTN treatment, increased to 6.7% day^{-1} and in the STN it decreased to 5.0% day^{-1}. At the end, shrimps of the LTN and STN treatments reached weights of 8.46 and 6.72 g and had productivities of 1287 and 1015 kg ha^{-1}, respectively. Shrimps reared in nurseries for long periods showed growth and survival rates similar to those obtained using conventional management practices in grow out structures.

Keywords: *Litopenaeus vannamei*, culture density, grow-out, long confinement, subtropical shrimp culture.

Almacenamiento a largo plazo y el crecimiento compensatorio de camarón blanco *Litopenaeus vannamei* en estanques de acuicultura

RESUMEN. Se analizó el efecto del confinamiento de camarones en una situación de alta densidad de población en una pre-engorda, sobre su crecimiento a largo plazo en los estanques de engorde. Dos estanques de pre-engorda con densidad de 2,000 camarones m^{-2} fueron sembrados en dos momentos diferentes. La primera crianza de largo plazo (LTN) duró 144 días, y la tasa de crecimiento específico (SGR) de los animales fue 3,0% $día^{-1}$. La segunda crianza de corto plazo (STN) duró 18 días y el SGR fue 19,9% $día^{-1}$. En el mismo día, los camarones fueron trasladados a seis estanques en una densidad de 20 camarones m-², donde permanecieron durante 101 días. En la primera biometría, la SGR en el tratamiento LTN aumentó a 6,7% $día^{-1}$ y STN disminuyó a 5,0% $día^{-1}$. Al final, los camarones de los tratamientos LTN y STN llegaron a 8,46 y 6,72 g con productividad de 1.287 y 1.015 kg ha^{-1}, respectivamente. Los camarones cultivados en pre-engorda durante largos períodos presentaron crecimiento y supervivencia similares a los obtenidos mediante prácticas de manejo convencionales en los estanques de engorda.

Palabras clave: *Litopenaeus vannamei*, densidad de cultivo, engorde, confinamiento prolongado, acuicultura subtropical.

INTRODUCTION

In aquaculture farms, there is a constant need to improve stocking techniques that encourage the storage of larger and healthier juveniles. One of these techniques consists in the management of post-larvae in nurseries for a certain time period and later transferring juveniles to grow out structures (Garza de Yta *et al*., 2004). The use of nurseries allows the storage of larger shrimps, which are more tolerant to environmental changes, in ponds, resulting in higher growth rates and also reducing the period of culture (Sturmer *et al*., 1992; Cohen *et al*., 2005; Cavalli *et al*., 2008; Mishra *et al*., 2008). In addition, another advantage is an increase in the favorable period for shrimp culture in the subtropics, where it is typically limited due to seaso-

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nal low temperatures in these areas. The increase of this period takes place with the storage of early post-larvae in tanks inside greenhouses, which enables the transfer of larger animals to the ponds for growth when temperatures are more appropriate (Kumlu *et al*., 2010). The reduction in time needed for grow out could increase the number of harvests, as well as extend the duration of grow out in a single crop; in both cases there is the potential for incremental productivity. In addition, this procedure enhances biosecurity because it has been recognized as an important tool in minimizing the risk of disease introduction and reducing mortality in farms already infected (Samocha *et al*., 2000; Fegan & Clifford, 2001).

Shrimps usually remain in nurseries for 10 to 25 days (Stern & Letellier, 1992) in some cases, up to 71 days (Mishra *et al*., 2008). In this period, it is recommended that the animals are fed at frequent intervals and the water quality parameters are strictly monitored. However, situations may occur in which the permanence of juvenile shrimps for more prolonged periods in nurseries is required. There are some problems, such as the reduction of growth for shrimp that are still in grow out ponds (low temperatures, high stocking densities), commercialization problems of shrimps ready for sale, structural problems in the facilities, the occurrence of disease in ponds, and stocking juveniles to overwinter.

There is another interesting issue in terms of farm management as a result of the use of nurseries. During the first weeks after transfer to grow out ponds, it is usual that shrimp present high growth values. This is known as "compensatory growth," a term used to describe situations in which organisms achieve accelerated growth after suffering some stressful factor, such as starvation, food and protein restriction, or hypoxia, and then returning to their normal condition, (Wilson & Osbourn, 1960; Wu *et al*., 2001; Ali *et al*., 2003; Zeigler, 2012). Since the 1970s, studies on compensatory growth in aquatic animals have been intensified because in addition to its ecophysiological importance, there are also implications for aquaculture. For example, the possibility to decrease the amount of food supplied resulting in a reduction in the cost of purchasing inputs and manpower, as well as a reduction in effluent emissions during the period of restricted food (Jobling *et al*., 1994; Hayward *et al*., 1997). However, although there are several studies on this theme, the most common target species are fish (Wilson & Osbourn, 1960; Jobling *et al*., 1994; Ali *et al*., 2003), while only a few studies use shrimps (Wu *et al*., 2001; Wei *et al*., 2008; Wasielesky *et al*., 2013).

The present study was conducted to analyze the possibility of compensatory growth of *L. vannamei* stocked in grow out ponds after a short and long period time nursery phase.

MATERIALS AND METHODS

The experiment was conducted in the facilities of the Marine Station of Aquaculture, Institute of Oceanography of Federal University of Rio Grande (FURG), Rio Grande do Sul, Brazil (32°12'15"S, 52°10'41"W). The experimental period lasted 245 days and was carried out in two steps - the nursery phase and the grow-out phase. In the first step, post-larvae (PL20) were stocked in two different seasons: winter, during which the shrimps were maintained for 144 days (treatment (LTN) 'Long Term Nursery'); and spring in which post-larvae (PL20) were maintained for 18 days (treatment (STN) ‘Short Term Nursery’). For this phase, two tanks (area of 20 m²) in a greenhouse were used; the stocking density was 2000 shrimp m^{-2}, the water exchange was 25% per day and the animals were fed *ad libitum* using commercial extruded feed (40% crude protein, 10% humidity, 7.5% ethereal extract, 5.0% fibrous matter, 13% mineral matter).The feed were supplied in feeding trays to evaluate and correct the amount of food to be provided. In the second step, shrimps from both nurseries were transferred to six HDPE lined ponds, each pond with an area of 600 m² and average depth of 1.0 m, with a stocking density of 20 shrimp m^{-2}. The grow-out period in the ponds lasted 101 days.

The temperature, dissolved oxygen, salinity and pH were monitored daily using the YSI 556 multiparameter device. The water transparency was measured every three days using a Secchi disk. Total ammonia nitrogen (UNESCO, 1983) was sampled weekly. The water exchange in the ponds was 1.0% per day. Commercial extruded feed (35% crude protein, 10% humidity, 7.5% ethereal extract, 5.0% fibrous matter, 13% mineral matter) was provided twice a day. The feeding rate was equivalent to 4.0% of the estimated biomass in the first half of the experiment and 3.0% in the final half; also a tray was used for feed control and to observe the shrimp in ponds. Samples were collected every 14 days for evaluation of shrimp growth. After each sample, the amount of feed was adjusted according to the mean weight and the estimated biomass. In the final sample, average weights, weekly growth rate, survival rate, productivity, specific growth rates (SGR) and feed conversion rate (FCR) were determined.

The specific growth rate was calculated using the following formula:

$$SGR\ (\%\ day^{-1}) = 100 \times (\ln W_F - \ln W_I)/T$$

where, W_F e W_I are the live final weight and initial weight (g) of shrimps inside a time interval (days).

The feed conversion rate was calculated using the following formula:

FCR = amount of feed/total biomass produced

The data were analyzed using the Student-*t* test, where ($P < 0.05$) values were considered indicative of significant differences. All tests were conducted after confirmation of normal distribution of the data (Kolmogorov-Smirnov's test) and homoscedasticity of variances (Levene's test). Before analysis of shrimp survival, the data were transformed into arcsine square root values.

RESULTS

Nursery period

The average temperature of the water in the LTN nursery was 18.9°C (9.5°C minimum and 24.1°C maximum). The average temperature of the water in the STN treatment was 22.9°C (21.3°C minimum and 24.1°C maximum). The salinities of the LTN and STN treatments were 28.2 and 29.0, respectively. There were no significant differences ($P > 0.05$) between treatments. The average pH values of the LTN and the STN treatments were 7.70 and 7.85, respectively. No significant differences ($P > 0.05$) were observed between treatments. The average values of ammonia in the LTN and STN treatments were 0.45 and 0.31, respectively. Additionally, no significant differences ($P > 0.05$) were found. The results of the zootechnical performances obtained during the nursery phase for both treatments are shown in Table 1.

Table 1. Days in the nursery, average initial and final weights, survival rates, weekly growth, and specific growth rates (SGR), productivity and feed conversions apparent of *Litopenaeus vannamei* post-larvae in treatment long term nursery (LTN) and short term nursery (STN).

	Nursery period	
	LTN	STN
Nursery days	144	18
Initial average weight (g)	0.008	0.010
Final average weight (g)	0.600	0.360
Survival (%)	79.7	93.0
Weekly growth (g)	0.03	0.14
SGR (% day^{-1})	2.98	19.91
Productivity (kg m^{-2})	0.96	0.67
Feed conversion rate	1.5	1.3

Grow-out period

The average values of the water quality parameters did not differ significantly ($P > 0.05$) between treatments. Values are shown in Table 2. The results of the zootechnical performance of shrimps in LTN and STN treatments are shown in Table 3. In the first phase (nursery), the shrimps had lower growth rates, mainly due to the high densities supported. In the second phase, the shrimps were transferred to grow out ponds, which resulted in higher growth values (Fig. 1). The values of the specific growth rate (SGR) in the treatments during the experimental period (nursery and grow-out) are shown in Figure 2.

DISCUSSION

Nursery period

Throughout the nursery period, the water quality parameters, except for temperature, were maintained within the ideal ranges for growth and survival of *L. vannamei* (Van Wik & Scarpa, 1999). Studies have shown that temperature affects the behavior of penaeid shrimp in subtropical areas. During the winter, shrimp become lethargic and remain buried in the substrate most of the time; this behavior reduces their food intake and growth rate (Soares *et al*., 2012). Analyzing the effect of temperature on the larval development of shrimp *Farfantepenaeus californiensis*, Villarreal & Llamas (2005) concluded that the duration of each larval stage was inversely related to temperature. Kumlu & Kir (2005) evaluating food consumption for shrimp *P. semisulcatus* in relation to molt stage and survival during winter, observed a trend of reduced specific growth rate (SGR) with the decrease of temperature. In the current study, the difference in SGR between the two treatments clearly demonstrates the influence of a high stocking density but is primarily heightened by the low temperatures, which contribute to the low growth of shrimp in the LTN treatment compared to the STN treatment.

In nurseries, the survival must be higher than 70% to ensure that this strategy is effective (Stern & Letellier, 1992; Sturmer *et al*., 1992; Moss & Moss, 2004). However, high stocking densities in nurseries tend to negatively affect the growth and survival of shrimps. The crowding associated with high stocking densities generates adverse behaviors, such as aggression and cannibalism that rapidly degrade water quality and increase the accumulation of undesirable sediments (Kumlu & Kir, 2005). The shrimps in the STN treatment remained stocked for just 18 days and this resulted in a higher survival rate. Nevertheless, the survival of the shrimps in treatment LTN was high,

Table 2. Average values (±standard deviation) of water quality parameters of ponds stocked with *Litopenaeus vannamei* for long term nursery (LTN) and short term nursery (STN) treatments during the grow-out period. There were no significant differences between treatments ($P > 0.05$).

	LTN	STN
Morning temperature (°C)	24.9 ± 1.6	24.8 ± 1.6
Afternoon temperature (°C)	27.2 ± 1.8	27.4 ± 1.7
Morning dissolved oxygen (mg L^{-1})	5.76 ± 2.17	5.81 ± 1.56
Afternoon dissolved oxygen (mg L^{-1})	8.19 ± 2.45	8.18 ± 1.54
pH morning	7.99 ± 0.21	8.14 ± 0.27
pH afternoon	8.12 ± 0.18	8.29 ± 0.23
Salinity	34 ± 2.5	36 ± 2.4
Transparency (cm)	45 ± 21	51 ± 27
Total ammonia (mg L^{-1})	0.46 ± 0.32	0.52 ± 0.46

Table 3. Results of zootechnical performance of shrimps: grow out days, initial and final weight, survival, weekly growth, specific growth rate (SGR), productivity and apparent feed conversion of *Litopenaeus vannamei* in long term nursery (LTN) and short term nursery (STN) treatments in the grow-out period. *Superscript (a and b) indicate significant differences ($P < 0.05$).

	Grow out period	
	LTN	STN
Initial weight (g)	0.60 ± 0.31	0.36 ± 0.09
Final weight (g)	8.46 ± 1.56[a]	6.72 ± 1.42[b]
Survival (%)	76.0 ± 9.3	75.9 ± 14.0
Weekly growth (g)	0.54 ± 0.3	0.42 ± 0.3
SGR (% day^{-1})	2.62 ± 0.3	2.90 ± 0.05
Productivity (kg ha^{-1})	1287[a] ± 182	1015[b] ± 125
Feed conversion rate	1.66 ± 0.14	1.50 ± 0.07

especially in light of the longer confinement time and the low temperatures noted in this treatment.

Grow out period

The values of water quality registered did not differ significantly ($P > 0.05$) between treatments and apparently did not affect shrimp growth in the grow-out period because the growth rates remained within the optimal range for penaeid shrimp (Van Wyk & Scarpa, 1999).

Several advantages are related to the use of nurseries (Garza de Yta *et al*., 2004). However, there is little information about the effect of the time of shrimp permanence in the nursery and subsequent performance in the grow-out period. In this study, at the time of transfer from the nursery to the grow-out ponds, the average shrimp weights were significantly different ($P < 0.05$) between the treatments. However, there is a common practice of using animals with a wide size range in specific experiments. For example, Williams *et al*. (1996) comparing the growth of shrimps *Penaeus setiferus* and *P. monodon* in ponds have used average initial weights of 0.24 and 0.12 g, respectively. Garza de Yta *et al*. (2004) compared the performance of shrimps that were stocked directly with shrimps that remained 10 and 20 days in nurseries. Zelaya *et al*. (2007), comparing the growth of *L. vannamei* juveniles stocked at three different ages, simultaneously populated shrimps with initial weights of 0.001 g (direct stocking), 0.01 g (14 days in a nursery) and 0.016 g (21 days in a nursery). In the present study, although the average weight was smaller in the STN treatment, the values of weekly growth and specific growth rate (SGR) were higher at the time of transfer, demonstrating that density and prolonged culture time strongly affected the growth of shrimp in the LTN treatment.

The survival rates of shrimp in both treatments did not show significant differences ($P > 0.05$). Barbieri & Ostrensky (2002) reported a survival rate of approximately 65% in *L. vannamei* commercial pond farming in Brazil. In this study, the feed conversion rates did not show statistically significant differences between LTN and STN treatments (1.66 and 1.50, respectively).

In semi-intensive production systems, the values of weekly growth can vary between 0.80 g to 0.94 g (Sandifer *et al*., 1993; Garza de Yta *et al*., 2004). In the same ponds used in this work, Peixoto *et al*. (2003), comparing growth between species, achieved average weekly growth of 0.84 g for *L. vannamei* and 0.78 g for *Farfantepenaeus paulensis* using stocking densities of 15 shrimp m^{-2}. In the present study, the weekly growth rates in both treatments were lower than the values reported above. The lower growth may be due to temperature values lower than the ideal (between 28 and 32°C) (Van Wik & Scarpa, 1999). However, during the first weeks after the transfer, growth rates were

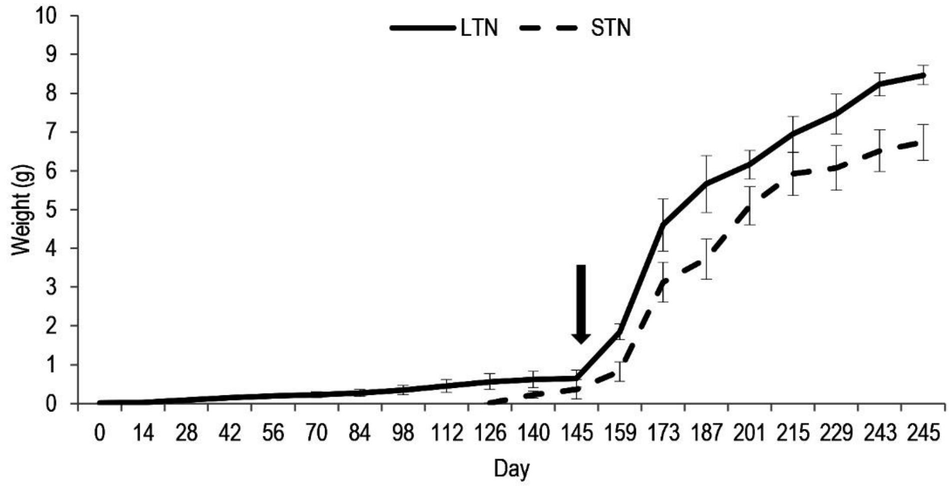

Figure 1. Shrimp growth in LTN (long term nursery) and STN (short term nursery) treatments during the complete experimental period. The arrow indicates the day of transfer from nursery to grow out ponds (day 144°).

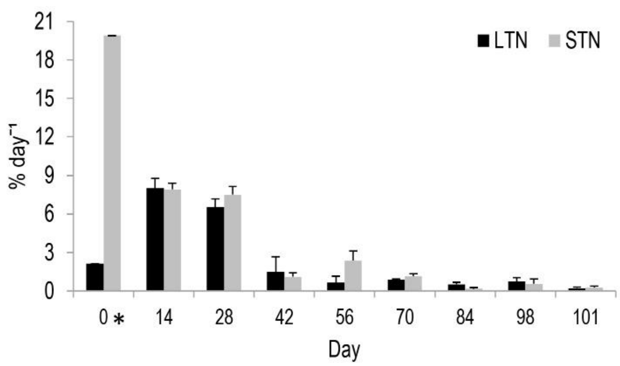

Figure 2. Specific growth rates (SGR) of *Litopenaeus vannamei* in treatments LTN (long term nursery) and STN (short term nursery) during the experimental period (nursery and grow out). *The SGR of day 0 (zero) corresponds to the average values during the nursery period for each treatment.

higher than average, especially in the LTN treatment (weekly growth of 1.0 g in the first month after transfer). This high growth rate suggests that this species achieved compensatory growth, a term used to describe an acceleration of growth due to the return of favorable conditions after a period of stress (food restriction or some other unfavorable environmental condition). Likewise, the growth can be identified as being significantly higher than the growth in the STN treatment, in which shrimp did not experience an induced stressful condition (Jobling *et al*., 1994; Ali *et al*., 2003; Nicieza & Alvarez, 2009). Moss *et al*. (2005) in an experiment on partial harvests of *L. vannamei* at high densities, achieved growth values of 0.95 g weekly until the first harvest, after which there was a weekly increas6e of 2.6 g for two weeks.

The specific growth rate (SGR) is a parameter typically used to identify compensatory growth. For example, Wu *et al*. (2001) used SGR in an experiment on food restriction and refeeding and also in an experiment on protein restriction of *Fenneropenaeus chinensis*; Ostrensky & Pestana (2000) used SGR to evaluate growth rates of *F. paulensis* in grow out ponds; Kumlu & Kir (2005) used SGR to evaluate growth and survival of *Penaeus semisulcatus* in temperate climates.

The SGR values showed that the compensatory growth occurred after the transfer from the nursery to grow out ponds. The growth of shrimp in the long term nursery was 2.1% day^{-1}. The growth of shrimp in the STN treatment was 19.9% day^{-1}. However, in the first biometry after the shrimps have been transferred to ponds, the SGR value increased to 7.9% day^{-1} in the LTN treatment. In the STN treatment, the SGR value for the nursery was 19.9% day^{-1} and decreased to 7.7% day^{-1} in first biometry in the ponds. From the second biometry until the end of the experiment, daily growth rates were similar between treatments. Normally, the absolute growth rates and the weight percentage are inversely proportional (Ostrensky & Pestana, 2000). This was evident in the present study, especially in the first biometry, when the compensatory growth was observed in shrimps of the LTN treatment.

Generally, the values of accelerated growth observed after a period of stress tend to decline to rates typical of control animals (Ali *et al*., 2003). Wu *et al*. (2001), studying the compensatory growth of *F. chinensis* under food restriction, found that after normalization of food supply the SGR of the treatment with dietary restrictions increased significantly (P <

0.05) compared to the control treatment. However, this increase in growth lasted only ten days. This result may have been determined by a physiological and behavioral characteristic of shrimps -the ecdisys activity. Bordner & Conklin (1981) have also detected this phenomenon for *Homarus americanus* juveniles. In crustaceans that have a large and inelastic exoskeleton, growth is a discontinuous process. There is a succession of molts (ecdisys) separated by intermolt periods throughout the life cycle. Food consumption by shrimp is greatly affected by pre and post molt phases, which are characterized by loss of appetite before and after the exchange of the exoskeleton (Hartnoll, 1982). Thus, it seems reasonable to infer that the duration of the compensatory growth response in this experiment was discontinued due to this characteristic of "voluntary starvation" during molt period.

The productivities were significantly different ($P < 0.05$) between treatments because the LTN treatment achieved productivity 26% greater than the STN treatment.

The results show an important capability of *L. vannamei* juveniles; even after a long period of confinement at high densities, when placed in a new less stressful environment, the juveniles react and present similar growth to individuals stocked in conventional farming protocols. It was also observed that storage in the nursery allows the maintenance of shrimps for long periods of time at high stocking densities, serving as a tool for reducing the growth rate without increasing productivity too much. The results confirm the possibility of maintaining shrimp in nurseries and fractionation of storages over time and achieve full compensatory growth.

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