

## A Comparative Study of Different Methods for Larval Selection on Survival Rate, Growth and Yield of Black Tiger Shrimp (*Penaeus monodon* Fabricius)

Supakorn Pattanavivat, Chalor Limsuwan, Niti Chuchird\* and Nitis Pattarakulchai

Department of Fisheries, Faculty of Fisheries, Kasetsart University,  
Bangkok 10900, Thailand

### ABSTRACT

Since 2000, black tiger shrimp (*Penaeus monodon*) production in Thailand has decreased because of the slow growth syndrome, poor survival and lack of predictability of postlarval (PL) performance in the growout phase. To solve these problems, many criteria were developed to assess larval quality before stocking in the growout pond. In this study, three methods for selecting PL were compared: group 1 using the Shrimp Biotech method; group 2, the Wanachsunthorn method; and group 3, conventional method (control). The shrimp were raised in growout ponds (15 ponds for each group) with low saline (1-5 ppt) water at a density of 80,000 PL per rai (1,600 m<sup>2</sup>) for 120 days. The results showed that the yield and survival rates of shrimp in group 1 were 987.60±170.25 kg/rai and 67.91±10.54% compared with 882.86±136.36 kg/rai and 66.18±10.34% in group 2. There were no statistically significant differences between the two groups. However, there were significant differences in the production and survival rates in group 3 which were 491.53±81.06 kg/rai and 45.24±7.11%, respectively. The results from this study indicated that evaluation of several criteria in groups 1 and 2 gave a better quality of shrimp seed and were major factors affecting the survival rate and production of cultured shrimp.

**Keywords :** Postlarvae, Shrimp Biotech method, Wanachsunthorn method, Black Tiger Shrimp (*Penaeus monodon*)

### INTRODUCTION

Black tiger shrimp used to be a major export item that earned 100 billion baht for Thailand yearly. But starting in 2002, the production of black tiger shrimp has dropped because harvests were not up to target. The shrimp grew slowly and many were small-sized (Limsuwan and Chanratchakool, 2004). Although stunted growth may result from a variety of causes, the quality of shrimp postlarvae (PL) is considered to be one of the most important factors influencing successful shrimp culture. Healthy PL reared in a good environment have the best chance of growing well and achieving good survival, harvests and profits (Saurabh *et al.*, 2006). Stress tests have been described and applied principally to distinguish between healthy and weak PL based on the exposure of shrimp to



environmentally adverse conditions (Maugle, 1988; Baybay, 1989; Tackarert *et al.*, 1989; Bauman and Jamadre, 1990; Bauman and Scura, 1990; Niestes, 1990; Gromez *et al.*, 1991). These include stress tests of salinity shock or salinity and temperature or to selected chemical solutions such as formalin (Briggs, 1992; Clifford, 1992; Tackaert *et al.*, 1989; Bauman and Jamandre, 1990). However, there is no reported evidence that the results of these stress tests are related to performance of PL during culture and growout (Aquacop *et al.*, 1991; Fegan, 1992; Samocha *et al.*, 1998; Racotta *et al.*, 2003).

Assessment PL health and fitness has become increasingly important and a major concern at both the research and production levels (Tayamen and Brown, 1999; Racotta *et al.*, 2003). Many different criteria have been used, including simply the stage of development from zoea 1 to PL, coloration, gut fullness, PL survival, muscle development, body deformity, presence of debris on setae, fatty acid profile composition, and the presence of virus occlusions has also been considered (Parado-Esteva, 1988; Browndy, 1992; Samacha and Lawrence, 1992; Banman and Jamandre, 1990; Villalon, 1991; Arellano, 1990; Gacutan and Grijado, 1996).

In this study three different methods commonly used in Thailand for evaluating the quality of shrimp PL were compared, determining any relationship to yield and growth rate of black tiger shrimp in the growout pond.

## MATERIALS AND METHODS

The *P. monodon* larvae used in this study were produced by a private hatchery located in Phuket province, Thailand. All the larval stage nauplii came from white spot syndrome virus (WSSV)-free broodstock. Larvae were reared until they reached PL15 and then randomly divided into three groups for evaluation of PL quality using three different methods. Group1 used the Shrimp Biotech method; group 2, the Wanatsunthorn method; and group 3, the conventional method (control), by observing only the normal appearance of the PL. Parameters used for each method are shown in Table 1.

PL from each group that passed the evaluation tests were transferred to a private shrimp farm in Ratchaburi province and stocked at a density of 80,000 PL/ 1,600 m<sup>2</sup> in 45 earthen ponds, with 15 ponds for each group. The ponds' areas ranged from 4,800-6,400 m<sup>2</sup> (3-4 rai). Salinity during the culture period ranged from 3-5 parts per thousand (ppt). Shrimp were fed with a commercial pelleted feed four times daily and feed quantity was adjusted following Limsuwan and Chanratchakool (2004). After 120 days the shrimp were harvested. Subsequently, shrimp from each pond were divided into two body weight groups: normal shrimp of more than 6 g and stunted shrimp under 6 g. Means and standard deviations of production, weight gain, FCR and survival rate of the shrimp from the three groups were calculated and compared using a one way analysis of variance (ANOVA).

**Table 1.** Comparison of three methods for selection high-health PL in this study.

<b>Criteria</b>	<b>Shrimp Biotech method</b>	<b>Wanachsunthorn method</b>	<b>Conventional method</b>
WSSV-free	Using PCR method	Using PCR method	Using PCR method
Sight inspection	1. Color condition. 2. Gut fullness. 3. Uniform size. 4. Swimming activity and behavior.	1. Condition of antennules 2. Coloration of uropods 3. Swimming activity and behavior	Same as Shrimp Biotech method
MBV	5. Detection of occlusion bodies with light microscope	no	no
Condition of the hepatopancreas	6. Coloration and size of the hepatopancreas	no	no
Lipid vacuoles	7. The amount of lipid vacuoles	no	no
Physical deformities	8. Necrosis and physical deformities of appendages	no	no
External parasites	9. Observation of external parasites	4. Observation of external parasites	no
Muscle to gut ratio	10. Observation of muscle to gut ratio (4:1)	5. Observation muscle to gut ratio (4:1)	no
Bacterial count on TCBS	11. <i>Vibrio</i> count (yellow colony 100 and green colony < 10)	no	no
Antibiotic residues	12. Detection of antibiotic residues	no	no
Average body weight	13. Average body weight at PL <sub>15</sub> stages	no	no
Stress test	Salinity stress test	6. Formalin stress test	no



Table 2. Statistical differences between the production of selected PL by three methods.

Parameter	Method	Average
Average weight (g)	Shrimp	17.1267 <sup>a</sup> (± 2.65455)
	BiotechWanatsunthorn	16.1160 <sup>a</sup> (± 2.32785)
	Conventional (control)	12.5260 <sup>b</sup> (± 2.61953)
Production (kg/1,600 m <sup>2</sup> )	Shrimp	987.6000 <sup>c</sup> (± 170.75873)
	BiotechWanatsunthorn	882.8667 <sup>c</sup> (± 136.36761)
	Conventional (control)	491.5333 <sup>d</sup> (± 81.06511)
Percent of normal shrimp after harvest (weight > 6 g)	Shrimp	94.8133 <sup>e</sup> (± 2.62811)
	BiotechWanatsunthorn	89.1333 <sup>f</sup> (± 2.70123)
	Conventional (control)	92.9000 <sup>e</sup> (± 2.17650)
Percent of stunted shrimp after harvest (weight < 6 g)	Shrimp	5.1533 <sup>g</sup> (± 2.63923)
	BiotechWanatsunthorn	10.8667 <sup>h</sup> (± 2.70123)
	Conventional (control)	7.1000 <sup>g</sup> (± 2.17650)
Weight gain/day (g)	Shrimp	0.1495 <sup>i</sup> (± 0.02405)
	BiotechWanatsunthorn	0.1373 <sup>i</sup> (± 0.02201)
	Conventional (control)	0.1039 <sup>j</sup> (± 0.02103)
Percent of survival rate (%)	Shrimp	67.9133 <sup>k</sup> (± 10.54716)
	BiotechWanatsunthorn	66.1867 <sup>k</sup> (± 10.34241)
	Conventional (control)	45.2400 <sup>l</sup> (± 7.11295)
Feed conversion rate (FCR)	Shrimp	1.3333 <sup>m</sup> (± 0.08398)
	BiotechWanatsunthorn	1.4313 <sup>m</sup> (± 0.13464)
	Conventional (control)	2.1793 <sup>n</sup> (± 0.29436)

Mean values with different superscripts are significantly different (P<0.05)

## RESULTS AND DISCUSSION

The results are shown in Table 2. There were no statistically significant differences between the total production, average weight, weight gain/day, survival rate and FCR of the shrimp in groups 1 and 2. However, the results of these two groups were significantly higher than those of group 3. Groups 1 and 2 had similar results for most parameters except percentage of small-sized shrimp. In this study PL that passed the evaluation method in group 1 had a lower percentage of small-sized shrimp than group 2. This might have been because the Shrimp Biotech method (group 1) had more criteria for PL selection and was more concerned with the condition of the hepatopancreas, especially the detection of *monodon* baculovirus (MBV) occlusions and the amount of lipid vacuoles. Flegel (2006) suggested that even though MBV is not a serious pathogen for the black tiger shrimp (Liao *et al.*, 1992; Fegan *et al.*, 1991), it should be eliminated from the farming system because it is unlikely that shrimp could carry such heavy viral infections without any effect on the shrimp. Flegel *et al.* (2004) showed that the mean length of MBV infected shrimp was significantly shorter than that of uninfected shrimp from the same pond. This report supported our results in



that the shrimp in group 1 showed a higher percentage of normal shrimp (weight > 6 g) than in group 2, although there is very little information available regarding the coloration and the amount of lipid vacuoles in the hepatopancreas. Good quality PL should have a dark color with star-like brown or dark pigmentation. The presence of a relatively large hepatopancreas with a large amount of lipid vacuolation is considered to be a sign of good health. PL with a small hepatopancreas containing few lipid vacuoles is a sign of underfeeding (Saurabh *et al.* 2006).

The occurrence of deformities of appendages and average body weight of PL were also criteria for PL selection in group 1. Healthy PL are generally more aggressive in searching for food and have a better chance of survival in the growout ponds than the deformed PL (Vitalon, 1991; Clifford, 1992; Smith *et al.*, 1992; Saurabh *et al.*, 2006). Postlarval size (length, wet weight) is a direct indicator of growth and thus reflects the degree of development at a given moment. Increased growth and reduced variability in size during PL stages has in turn been related to further growth to juvenile stages (Racotta *et al.*, 2003; Castille *et al.*, 1993; Samocha *et al.*, 1989; Bray and Lawrence, 1992).

In addition to the criteria described above, the Shrimp Biotech method (group 1) had two more criteria for selection of good quality PL. One was *Vibrio* spp count on TCBS agar. Another one was antibiotic residues check. *Vibrio* spp, have been reported to cause larval mortalities in hatcheries and pond reared black tiger shrimp (Sunaryanto and Mariam, 1986; Baticados *et al.* 1990; Lavilla-Pitogo *et al.*, 2000). It is a common practice for the farmer to give antibiotic dip treatment for broodstock and larvae in hatcheries to reduce the shrimp mortality rate (Otta *et al.*, 2000; Liu *et al.*, 1997). Moriarty (1999) suggested that many farmers also use large quantities of antibiotics as prophylactics, even when pathogens are not evident. This has led to an increase in more virulent pathogens because of the transfer of genes for antibiotic resistance. Considering this aspect, using good hatchery management practices will improve the quality of PL produced.

It could be concluded that the quality of shrimp seed is a major factor affecting production in shrimp culture. The larval selection methods investigated in this study could be applied to establish an effective system for producing and distributing good quality PL to the farmer. Specific pathogen-free and genetically improved broodstock will also be required for the future successful development of commercial black tiger shrimp farming.

## ACKNOWLEDGEMENTS

The authors would like to thank the National Research Council of Thailand for financial support.



## LITERATURE CITED

- Arellano, E. 1990. Fatty acid composition of wild and culture *Penaeus vannamei* as a method to evaluate postlarvae quality, pp. 50/T 8.3. In Abstracts World Aquaculture 90, National Research Council Canada. Ottawa, Ontario, Canada
- Aquacop, G. Le Moullac and D. Damez. 1991. Modelisation de la resistance aux chocs de salinite des postlarvae de *Penaeus vannamei*. *Aquat. Living Resour.* 4: 169-173.
- Baticados, M.C.L., C.R. Lavilla-Pitogo, E.R. CryLacierda, L.D. da la Pena and N.A. Sunaz. 1990. Studies on the chemical control of luminous bacteria *Vibrio harveyi* and *Vibrio splendidus* isolated from diseased *Penaeus monodon* larvae and rearing water. *Dis. Aquat. Org.* 9:133-139.
- Bauman, R.H. and D.R. Jamadre. 1990. A practical method for determining quality of *Penaeus monodon* (Fabricius) fry for stocking in grow-out ponds, pp.124-137. In M.B. New, H. Saram and T. Singh, eds. Technical and Economic Aspects of Shrimp Farming. Proceedings of the Aquatech' 90 conference, 11 June -14 June 1990, Kuala Lumpur, Malaysia INFOFISH.
- Bauman, R.H. and E.D. Scura. 1990. Determining the quality of *Penaeus monodon* postlarvae, pp. 101/T 30.2. In Abstracts World Aquaculture 90, National Research Council Canada. Ottawa, Ontario, Canada. .
- Baybay, L. 1989. The Effect of Temperature and Salinity on *Penaeus monodon* Postlarvae with Observations on Commercial Hatchery Techniques in Relation to Post-larval Quality. Master's thesis. Asian Institute of Technology, Bangkok, Thailand.
- Bray, W.A. and A.L. Lawrence. 1992. New concepts in seedstock production : learning to determine quality, pp. 58-69. In Memorias, Simposium Internacional Sobre Laboratorios de Produccion de Postlarvas de Camaron, 5-7 December 1991, Mazatlan, Sinaloa, Mexico. Direccion General de Acuacultura, Secretaria de Pesca
- Browndy, C.L. 1992. A review of the reproductive biology of *Penaeus* species : perspectives on controlled shrimp maturation systems for high quality nauplii production, pp. 22-51. In J. Wyban, ed. Proceedings of the Special Session on Shrimp Farming. World Aquaculture Society. Baton Rouge, LA, USA.
- Briggs, M.R.P. 1992. A stress test for determining vigour of post-larval *Penaeus monodon* Fabricius. *Aquacul. Fish. Manage.* 23: 633-637.
- Castille, F.L., T.M. Samocha, A.L. Lawrence, H. He, P. Frelier and F. Jaenike. 1993. Variability in growth and survival of early postlarval shrimp (*Penaeus vannamei*, Boone 1931) *Aquaculture* 113: 65-81.
- Clifford, H. C. 1992. Marine shrimp pond management : a review, pp. 110-137. In J. Wyban, ed. Processings of the Special Session on Shrimp Farming. World Aquaculture Society, Baton Rouge, LA, USA.
- Fegan, D.F., T.W. Flegel, S. Sriurairatana and M. Waiyakrukruha. 1991. The Occurrence, development and histopathology of monodon baculovirus in *Penaeus monodon* in southern Thailand. *Aquaculture* 96 : 205 - 217.



- Fegan, D.F. 1992. Recent developments and issues in the penaeid industry, pp. 55-70. *In* J. Wyban, ed. Proceedings of the Special Session on Shrimp Farming. World Aquaculture Society. Baton Rouge, LA, USA.
- Flegel, T.W., L. Niesen, V. Thamavit, S. Kongtim and T. Pasharavwipas. 2004. Presence of multiple viruses in non-diseased, cultivated shrimp at harvest. *Aquaculture* 240: 55-68.
- Flegel, T.W. 2006. Detection of major penaeid shrimp viruses in Asia, a historical perspective with emphasis on Thailand. *Aquaculture* 258: 1-33.
- Gacutan, R.O. and G.A. Grijado. 1996. Revision of the fry quality assessment scheme presently *In* Book of Abstracts, Second International Conference on the Culture of Penaeid Prawns and Shrimps. Aquaculture Department, Southeast Asian Fisheries Development Center, Ilo-ilo City, Philippines.
- Gomez, R.O., J.M. Rodriguez and J. Morales. 1991. Stress-tests : a practical tool to control postlarval shrimp quality, pp. 358-360. *In* P. Lavens, P. Sorgeloos, E. Jaspers, F. Oliver, eds. Larvi'91 : Fish and Crustacean Larviculture Symposium, 27-30 August, Gent, Belgium. (Special Publication) European Aquaculture Society.
- Karunasagar, I., R. Pai, G.R. Malathi and I. Karunasagar. 1994. Mass mortality of *Penaeus monodon* larvae due to antibiotic resistant *Vibrio harveyi* infection. *Aquaculture* 128:203-209.
- Lavilla-Pitogo, C.R., M.C.L. Baticados, E.R. Cruz-Lacierda and L.D. da la Pena. 1990. Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. *Aquaculture* 91:1-13.
- Liao, I.C., M.S. Su and C.F. Chang. 1992. Diseases of *Penaeus monodon* in Taiwan : a review from 1977 to 1991, pp. 113-137. *In* W. Fulks and K.L. Main, eds. Diseases of Cultured Penaeid Shrimp in Asia and the United States. Oceanic Institute, Honolulu, HI.
- Limsuwan, C. and P. Chanratchakool. 2004. Shrimp Farming Industry in Thailand. National Research Council of Thailand, Bangkok, Thailand. 206 p.
- Liu, P.C., K.K. Lu and S.N. Chen. 1997. Susceptibility of different isolates of *Vibrio harveyi* to antibodies. *Microbios* 91:368-369.
- Maugle, P. 1988. Post-larvae shrimp mortality study. *Artemia Newsletter* 8.
- Moriarty, D.J.W. 1999. Disease Control in Shrimp Aquaculture with Probiotic Bacteria. *In* C.R. Bell, M. Brylinsky and P. Johnson-Green, eds. Proceedings of the 8<sup>th</sup> International Symposium on Microbial Ecology. Atlantic Canada Society for Microbial Ecology, Halifax, Canada.
- Niestes, A.D. 1990. The Effect of Feed Types/Forms on the Growth and Quality of *Penaeus monodon* Postlarvae. Master's thesis. Asian Institute of Technology, Bangkok, Thailand.
- Otta, S.K., I. Karunasagar and I. Karunasagar. 2001. Bacteriological study of shrimp, *Penaeus monodon* Fabricius, hatcheries in India. *J. Appl Ichthyol.* 17:59-63.
- Parado-Esteva, F.D. 1988. Selection transport and acclimation of prawn fry, pp.81-85. *In* Y.U. Chiu, L.M. Santos and R.O. Joliano, eds. Technical Considerations for the Management and Operation of Intensive Prawns Farms. Aquaculture Society, Ilo-ilo City, Philippines.

- Racotta, I.S., E. Palacios and A.M. Ibarra. 2003. Shrimp larval quality in relation to broodstock condition. *Aquaculture* 227 : 107-130.
- Samocha, T.M., H. Guajardo, A.L. Lawrence, F.L. Castille, M. Speed, D.A. McKee and K.M. Page. 1998. A simple stress test for *Penaeus vannamei* postlarvae. *Aquaculture* 165: 233-242.
- Samocha, T.M., N. Uziel and C.L. Browdy. 1989. The effect of feeding two prey organisms, nauplii of *Artemia* and rotifers, *Brachionus pelicanilis* (Muller), upon survival and growth of larval marine shrimp, *Penaeus semisulcatus* (de Haan). *Aquaculture* 77: 11-19.
- Samacha, T.M. and A.L. Lawrence. 1992. Shrimp nursery systems and management, pp. 87-105. In J. Wyban, ed. Proceedings of the Special Session on Shrimp Farming. World Aquaculture Society. Baton Rouge, LA, USA.
- Saurabh, S., V. Kumar, S. Karanth and G. Venkateshwarlu. 2006. Selection of high-health postlarvae: A prerequisite for sustainability of the Indian shrimp industry. *Aquaculture Asia* 11(2) : 4-9.
- Smith, L.L., T.M. Samacha, J.M. Biedenbach and A.L. Lawrence. 1992. Use of one-liter Imhoff cones to optimize larviculture production, pp. 287-300. In A.W. Fast and L.J. Lester, eds. Marine Shrimp Culture : Principles and Practices. Elsevier, Amsterdam, Netherlands.
- Sunaryanto, A. and A. Mariam. 1986. Occurrence of a pathogenic bacteria causing luminescence in penaeid larvae in Indosian hatcheries. *Bull. Brackishwater Aquaculture Dev. Centre* 8:64-70.
- Tackaert, W., P. Abelin, P. Dhert and P. Sorgeloos. 1989. Stress resistance in postlarvae penaeid shrimp reared under different feeding procedures. *J. World Aquac. Soc.* 20:74A.
- Tayamen M. and J.H. Brown. 1999. A condition index for evaluation larval quality of *Macrobrachium rosenbergii* (de Man 1879). *Aquac. Res.* 30: 917-922.
- Villalon, J.R. 1991. Practical Manual for Semi-intensive Commercial Production of Marine Shrimp. Texas A&M University Sea Grant Program, Galveston, TX.