Aquaculture and Marine Biotechnology

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Evidence of the existence of resistance/tolerance to WSSV in *Penaeus monodon*

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1. Introduction

Indian seafood export stands at 3, 43,031 tones worth Rs. 5,117 crores per year. As foreign exchange winner, shrimp played a significant role by contributing 49 to 76% (Rs.3645 crores) to total export during 1978-2003. During 1977-88, the shrimp export was just 51,000-55,736 tones, however, from 1988-89 to 2002-2003 a spectacular growth could be recorded culminating at 1, 34,815 tones. This growth was mainly due to the contributions from aquaculture sector, which in 2002—2003 alone amounted to 1, 15,320 tones. The shrimp production from wild has been gradually declining from 50,000 tones during the eighties to 19,495 tons in 2002-2003 due to over exploitation of commercial species. The Indian tiger shrimp *Penaeus monodon* is the principal species which contributes to the cultured shrimp production in the country.

The shrimp culture practice has been extended to vast areas along the Indian coast. To support this magnificent industry 232 shrimp hatcheries have been established, especially along the East Coast. Meanwhile the industry had to face the biggest calamity in its history during 1995-96 in the form of dreaded white spot syndrome virus which continued without much relief. To address this issue and stabilize the industry production of specific pathogen free shrimp has been identified as the prime option. Nevertheless, hatcheries are compelled to depend on wild gravid females even today due to the failure of brood stock management system in captivity as well as the non-response of wild females to eyestalk ablation. The situation is still serious that there is inconsistent availability of gravid females from wild resulting in inadequate production of seed at appropriate time. Availability of limited number of gravid females from wild and the exorbitant cost (Rs.15, 000 to 70,000/piece) force the hatchery allow spawning of WSSV infected gravid females which would rekindle the emergence of epidemics. It has been established that this is the practice which keeps the culture environment always under the threat of disease out breaks (Mohan et al., 1997; Tsai et al., 1997). Hence, domestication shrimp is an absolutely essential requirement to produce specific pathogen-free brood stock/ disease resistant brood stock in captivity to put back the industry to its previous

comfortable position of the early nineties (Sakthivel and Ramamurthy, 2003). In this process, selective breeding is an essential component to achieve development of desired traits in the candidate species. Virus-resistant strains of *P.stylirostris* (Super Shrimp) that were developed through selective breeding in Venezuela without eyestalk ablation have assisted Mexico to reestablish itself as one of the top shrimp producing mariculture countries in Latin America (UJNR Technical Report No.28). Developments in penaeid broodstock and seed production technologies with an outlook for superior captive stocks were reviewed by Browdy (1998). The approach and initial results in developing a selective breeding programme for a SPF stock of *P. vannamei* was reported by Wyban (1992). Schultz (1986) described the protocol and guide lines for developing a commercial breeding programme for fish and shell fish. Lester (1999) gave an account on the best management practices for domestication of aquatic species with emphasis on *P.vannamei*.

2. Specific pathogen free versus disease resistant brood stock

Merits and demerits of Specific Pathogen Free (SPF) brood stock versus Disease Resistant (DR) brood stock of *P. monodon* are presented in the Table 1. Development of SPF brood stock in captivity involves very stringent management of environment to restrict the entry of pathogen into rearing system which can be designated in general as bio-security packages. Necessity of bio-security in shrimp research programmes was emphasized by Moss (2002), Schuur (2003) and Pruder (2004). Initially sea water is treated with disinfectants and subsequently precautionary measures are taken to arrest the entry of pathogen through water, feed and/or working personnels. Disease-free animals are selected and subsequently tested periodically for the existence of Specific Pathogens and those which test negative alone will be used in the development programme. Selection of more economically important traits such as faster growth, high reproductive performance, and good food conversion ratio (FCR) is also possible through this programme. Though SPF shrimp arrests the vertical transmission of diseases, it is susceptible to horizontal transmission. In the case of Disease Resistant brood stock, shrimps are challenged with the concerned pathogen to reveal the existence of resistance to the pathogen. In this case selection of only one trait, such as resistance / tolerance to the pathogen alone is possible and the other traits such as faster growth, high reproductive performance, and high FCR turns out to be non selective. However, they also become selective provided these are linked traits/genes. As SPF shrimp is susceptible to horizontal transmission, disease resistant shrimp ensures sustainable production, most suitable for Indian conditions.

Table 1. Specific Pathogen-Free broodstock versus Disease Resistant Broodstock

S.No.	Specific Pathogen-Free Broodstock	Disease Resistant Broodstock
1	Bio-security	Challenging with the concerned pathogen aimed to examine the prevalence of resistance against it
2	Selection of more economic traits-growth, high reproductive performance, and high Food Conversion Ratio(FCR) possible	Selection of one trait only-Resistance/Tolerance possible
3	Subject to disease manifestation in grow-out	Resists disease in grow-out
4	Yields high production with superior characteristic but susceptible to disease	Ensures sustainable production

3. Resistance/Tolerance for disease

In shrimp population subjected to pathogen in captivity/ culture system some or few of the animals survive (competent) and majority die with infection (Wang et al., 1998). Competent animals have some sort of internal mechanism called resistance/tolerance to fight against the pathogen and survive. This is observed in shrimp both at level and individual levels. Information from hatcheries reveals that most of the wild populations of *P. monodon* are carriers of WSSV and MBV, and a few individuals of the same population from the same location are disease free. Even in culture system when stock of mono culture is infected with WSSV some of the population do survive, which may be called resistance at individual level. When wild populations of different species at one location are tested for particular pathogen some are found carriers of the pathogen (Wang et al., 1998). They detected WSSV at three stages such as expression of clinical signs, PCR first stage amplification (1447bp product) and PCR second stage amplification (p41bp product). All cultured shrimp (*P. monodon, P. japonicus, P. pencilliatus* and *M. ensis*) were positive to all three stages; the wild shrimps, *Trachypenaeus curvirostris* and *M. ensis* were free from the virus where as *P. semisulcatus* was positive only at PCR second stage amplification; all wild crabs and lobsters were tested negative to the virus. In polyculture experiments also during disease outbreaks some species used to escape from the virus and others succumbed. This indicated the resistance/

tolerance to WSSV at species level. *P. semisulcatus* has been found resistant to WSSV compared to *P. monodon* (Authors experience). *P.stylirostris* used to be more tolerant to Taura Syndrome Virus than *P.vannamei.*

3.1. Experimental evidence for existence of resistance/tolerance at species level

An experiment on polyculture with three Indian cultivable Penaeids, namely tiger shrimp *P. monodon*, green tiger shrimp *P. semisulcatus* and Indian white shrimp *P.indicus* was conducted in a small pond of 0.08 ha area at the marine fish farm, Regional Centre of CMFRI, Mandapam with a stocking density of 5.9/m^2 and stocking ratio of 8.3:6.4:1 for *P.semisulcatus, P.monodon,* and *P.indicus,* respectively. Seeds of the green tiger shrimp and tiger shrimp were produced in the backyard hatchery of Regional Centre of CMFRI and seed of Indian white shrimp were collected from nearby lagoon. Shrimps were fed with commercial pelleted diet. After 70 days of culture the animals were challenged with WSSV and on observation of mortality the culture was terminated. Harvested shrimps were segregated species-wise and individually observed for infection by clinical signs. Only specimens of *P. monodon* were infected with WSSV but not the other two species. This experiment indicated that *P. semisulcatus* and *P.indicus* were resistant/tolerant to WSSV compared to *P. monodon*. Wang et al., (1998) found differential mortality rates in experimentally infected shrimps such as *Exopalaemon orientalis, Trachypenaeus curvirostris, Metapenaeus ensis, Macrobrachium sp.* and *Procambarus clarkia*. However, Rajendran et al., (1999) reported 100% cumulative mortality in experimentally infected *Penaeus monodon, P. indicus, P. semisulcatus, Metapenaeus monoceros* and *M.dobsoni* within 5-7 days when the animals were injected and 7-9 days when fed with infected tissue.

3.2. Experimental evidence for existence of resistance/tolerance at individual level

An experiment on domestication of tiger shrimp *P. monodon* was conducted during December 1998-September 2002. Two adult female *P.monodon* measuring 225 mm TL/120g and 245mm TL/135g were collected from Palk Bay, introduced in RMT and unilaterally ablated. Males were also introduced in equal number. Since mating did not take place, artificial insemination was carried out. PLs produced from these two spawners were reared up to adult in growout ponds for 150 days under similar conditions. Of these two populations, one showed faster growth from which 280 superior (growth) animals of both sexes were selected and transferred into 100 tones cement rectangular tank(10x5x2m) and reared for another 6 months. The brood stocks were fed with pelleted diet blended with vitamin C, Vitamin E, Ultramin,

fish oil and cod liver oil. Broodstocks were also given prophylactic bath treatment of Prefuran, OTC and formalin periodically. In January 1999 viable post larvae of 22,029 of F_2 generation were produced and 18000 PL_{17} were stocked in 0.15 ha growout pond. After 30 days, the F_2 population was infected with WSSV. Pond water was treated with $KMnO_4$ and left without further water management. After 88 days, 107 animals (58males+49females) survived in this pond; and were reared up to adult (100g size) in 100tones cement tank as done in the case of F_1 generation.

Broodstock of F_2 generation was segregated into two groups, one group stocked in RMT along with F_2 generation males to facilitate inbreeding, and the other group was stocked with males that were collected from Bay of Bengal off Visakhapatnam, to facilitate cross breeding. In total, 910 PL_{14} were produced from inbreeding experiment in January 2001 and reared up to adult in indoor fiber glass tanks (5 tones capacity). In March 2001, a total of 37,000 PL_{25} were produced from cross breeding experiment. Of these, 35,000 were stocked in two commercial growout ponds of 0.8 ha area. Commercial seed, which were stocked in other grow out ponds in the same farm, were experimentally infected with WSSV after 50 days. F_3 population survived up to 65 days with 50 % survival and attained the size of 134 mm TL/18g. The remaining 2,000 PL_{25} of cross breeding experiment were reared up to adult in indoor fiber glass tanks (5 tones capacity) and conducted breeding experiments to produce F_4 generation. F_3 adults that were tested for WSSV were positive only at PCR second stage amplication, indicating the vertical transmission of WSSV from F_2 adults, but due to resistance/tolerance at individual level these animals survived. During this domestication experiment it was observed that a few animals (0.6%) of F_2 generation population survived after infection with WSSV and they matured to produce F_3 generation. This indicated the existence of resistance/tolerance at individual level in *P. monodon*. Wang et al. (1998) conducted experimental WSSV infection in wild caught shrimp and observed mortality up to 18 days. All moribund animals during 18 days were positive for PCR first step where as survived animals were nested positive only after 18 days revealing the existence of resistance/tolerance to WSSV in survived animals.

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References

Browdy, C.L., 1998. Recent developments in Penaeid broodstock and seed production technologies: improving the out look for superior captive stocks. Aquaculture, 164: 3-21.

Lester, L.J., 1999. Best management practices for domestication of aquatic species with emphasis on *P.vannamei*. In: Proceedings from Fifth Ecuadorian Aquaculture Conference, Guayaquil, Ecuador. October, 1999 .

Mohan, C.V., Sudha, P.M., Shankar, K.M., Hegde, A.,1997. Vertical transmission of white spot syndrome baculovirus in shrimps-a possibility? Current Science, 74:109-110.

Moss, S.M., 2002. Marine shrimp farming in the Western Hemisphere: Past problems, present solutions, and future visions (abstract). Review. Fish. Sci.10:601-620.

Pruder, G.D., 2004. Biosecurity: application in aquaculture. Aqua. Eng. 32, 3-10.

Rajendran, K.V., Vijayan, K.K., Santiago, T.C., Krol, R.M., 1999. Experimental host range and histopathology of white spot syndrome virus (WSSV) infection in shrimp, prawns, crabs and lobsters from India. J.Fish Dis. 22:183-191.

Sakthivel, M., Ramamurthy, B., 2003. What ails shrimp farming and its future development? Fishing Chimes, 22: 33-36.

Schultz, F.T., 1986. Protocol and guide lines for developing a commercial breeding programme for fish and shell fish. Aquaculture, 57: 65-76.

Schuur, A.M., 2003. Evaluation of biosecurity applications for intensive shrimp farming. Aqua. Eng., 28:2-20.

Tsai, M.F., Lo, C.F., Liu, H.C., Liu, K.F., Kou, G.H., 1997. Detection of white syndrome virus (WSSV) in offsprings of a WSSV carrier brooder by *in situ* hybridization. In: Proceedings of the International Symposium on Diseases in Marine Aquaculture, October 1997, Japan

Wang, Y.C., Lo, C.F., Chang, P.S., Kou, G.H., 1998. Experimental infection of white spot baculovirus in some cultured and wild decapods in Taiwan. Aquaculture, 164:221-231.

Wyban, J.A., 1992. Approach and initial results in developing a selective breeding programme for a SPF stock of *P.vannamei*. In: Wendy Fulks, Kevan L.Main (Eds) Diseases of Cultured Penaeid Shrimp in Asia and the United States. Proc. of a workshop, April 27-30, 1992, Honolulu, Hawaii, p. 257-268.