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Selective Breeding and Development of Disease Resistant Broodstock of Black Tiger Shrimp *Penaeus monodon* Fabricius, 1798

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Abstract
The Indian tiger shrimp *Penaeus monodon* is the principal species being cultured. Of late hatchery sector is being dependent on wild gravid females due to non response of broodstock to eyestalk ablation. High cost of gravid females compelling the grow-out sector to stock pathogen carrier seeds. Hence domestication of tiger shrimp is essential to produce Specific Pathogen Free (SPF) broodstock / Disease Resistant (DR) broodstock. Merits and demerits of SPF versus DR broodstock are presented. Development of SPF broodstock involves stringent management of environment to arrest the entry of pathogens and more than one economic trait can be selected. Whereas in development of DR broodstock animals are challenged with the pathogen and a selection of other economic traits are less possible. Resistance in shrimp exists at the species level as well as individual level. Experiment on domestication of *P. monodon* in which programme was advanced up to F_3 generation has revealed the existence of resistance for WSSV at the individual level. Selective breeding programme for development of D R broodstock involves development of disease free base population, forming them into families, production of F_1 generation family wise through inbreeding, challenging each family with WSSV at 3-5 g size and rearing survived individuals up to 100 g size, production of F_2 generation by random inter crossing between families, advancing the programme up to F_5 generation. Development of DR broodstock is imperative to ensure sustainable shrimp production.

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1. Introduction

1.1 Shrimp culture and production

Indian seafood exports stand at 9,83,756 t worth Rs. 30,213.26 crores during 2013-14. Frozen shrimp is playing a significant role by contributing 64.1% (Rs.19, 368.3crores) to the total export value during 2013-14. During 1977-88, shrimp exports was at 51,000-55,736 t and from 1988-89 to 2002-03 a spectacular growth was recorded and reached 1, 34,815 t and by 2013-14 it has reached 3,01,435 t. This growth was mainly due to contribution from culture sector, which in 2002-03 amounted to 1, 15, 320 t and during 2013-14 it reached 2,20,978 t. The Indian tiger shrimp *P. monodon* was the principal species contributing cultured shrimp production until introduction of *P. vannamei* in 2009, being cultured in large scale along the Indian coast. Both *P. monodon* and *P. vannamei* has been contributing to shrimp production from the culture sector since 2010 and during 2013-14 *P. vannamei* contributed 1,75,071t and *P. monodon* contributed only 45,907 t (Marine Products Export Development Authority; http://www.mpeda.com/stat1314.pdf).

During 2012-13, *P. vannamei* was cultured in 22, 715 ha and produced 1,47, 516 t, resulting in

producing 6.49 t/ha. Where as *P. monodon* was cultured in 93,110 ha and produced 1,23, 303 t resulting in producing 1.32 t/ha. *P. vannamei* being exotic species, need more attention in the culture sector compared to that of native species *P. monodon.* However, *P. vannamei* is also subjected to White Spot Syndrome Virus, encountering difficulties to sustain.

1.2 Origin of the concept

This paper was presented by the corresponding author, as a resource person invited for Indo-Singapore Joint Workshop on "Frontiers in Aquaculture and Marine Biotechnology" held during 22nd-24th April 2004, at Avenue Regent, Ernakulum, India, organised by the Department of Biotechnology, Government of India, National Centre for Aquatic Animal Health, Cochin University of Science and Technology (CUSAT) and Central Marine Fisheries Research Institute.

1.3 Indian tiger shrimp Penaeus monodon

The culture practice of *P. monodon* has been extended to vast areas along the Indian coast due to establishment of as many as 232 shrimp hatcheries and consequent production of shrimp seed on large scale especially along the East coast. Unfortunately, shrimp culture industry-faced setback during 1995-96 due to the outbreak of dreaded white spot syndrome virus and since then the industry has faced this problem quite frequently. Of late, all the commercial hatcheries are depending on wild gravid females due to failure of broodstock management system in captivity as well as non-response of wild females to eyestalk ablation. Inconsistent availability of gravid females from the wild is the main reason for inadequate production of seed at the appropriate time. Availability of the limited number of gravid females from wild and the high cost (Rs.17, 000 to 56,000/piece; Miriam Paul et al., 2004) also compelling the hatchery sector to use pathogen carrier gravid females and culture sector to stock pathogen carrier seed that produced from those gravid females leading to vertical transmission and subsequent disease manifestation under stress conditions (Mohan et al., 1997; Tsai et al.,1997). Hence domestication of the tiger shrimp is essential to produce Specific Pathogen Free brood stock/Disease Resistant broodstock in captivity to revive the industry to its previous position in the early nineties by arresting disease manifestation (Sakthivel and Ramamurthy, 2003). Comparative study of the reproductive performance and White Spot Syndrome Virus (WSSV) status of black tiger shrimp (*Penaeus monodon*) collected from the Bay of Bengal by Debnath et al. (2014) also revealed the existence of negative correlation between the hatching rate and WSSV infection, and they suggested a better alternative could be to promote the use of domesticated *P. monodon* broods. Selective breeding is an essential component in a domestication programme to achieve the development of the desired traits of economic importance in targeted species. Virus-resistant strains of *P. stylirostris* (Super Shrimp) that were developed through selective breeding in Venezuela without eyestalk ablation, has assisted Mexico to reestablish itself as one of the top producing shrimp 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 an SPF stock of *P. vannamei* was reported by Wyban (1992). Schultz (1986) described the protocol and guidelines for developing a commercial breeding programme for fish and shellfish. Jim Lester (1999) gave an account of the best management practices for domestication of aquatic species with emphasis on *P. vannamei*. Pros and cons of selective breeding of shrimp for development of disease resistant broodstock were discussed by Moss and Doyle (2005). RGCA successfully developed 6th generation Specific Pathogen Free tiger shrimp (*Penaeus monodon*) during the Pilot scale operations of the project being carried out at OSSPARC facility, Gopalpur-on-Sea, Odisha, India (http://www.rgca.org.in/Tigershrimps.php).

2. Specific Pathogen Free (SPF) Broodstock versus Disease Resistant (DR) Broodstock

Merits and demerits of Specific Pathogen Free broodstock versus Disease Resistant broodstock of *P. monodon* are presented in the table, 1. Development of SPF broodstock in captivity involves very stringent management of environment to restrict the entry of pathogen into rearing system, i. e. Biosecurity. Necessity of bio security in shrimp research programmes was emphsised by Moss (2002), Schuur (2003) and Pruder (2004). Initially intake sea water is treated with disinfectant and subsequently precautions will be taken to arrest the entry of pathogen through the all sources like water, feed and by contamination through the working personnel. Disease free animals are selected and subsequently tested periodically for the existence of Specific Pathogens and only pathogen free animals are continued in the development programme. Selection of the most economically important traits like growth, high reproductive performance and low food conversion ratio (FCR) are possible for development of Superior shrimp. Though SPF shrimp will arrest the vertical transmission of diseases, it is susceptible to horizontal transmission of diseases. SPF shrimp with superior characteristics, fetches

Table 1: Specific Pathogen Free (SPF) broodstock versus Disease Resistant (DR) broodstock

S. No.	Specific Pathogen Free Broodstock (SPR)	Disease Resistant broodstock (DR)
1	The application of strict bio-security to arrest the horizontal entry of pathogens	Challenging with the concerned pathogen aimed to develop resistance against that
2	Selection of more economic traits-growth, high reproductive performance and low Food conversion ratio (FCR) are possible	Selection of one trait only-Resistance/Tolerance possible
3	Subject to disease manifestation in grow-out system	Resists disease in grow-out system
4	Yields high production with superior characteristics of growth and FCR but susceptible to disease	Ensures sustainable production

high production leading to more profits. In the case of Disease Resistant broodstock, shrimp is challenged with the concerned pathogen aimed to develop resistance to it. Here selection of only one trait, i.e. resistance /tolerance is possible and other economical traits like growth, high reproductive performance and low FCR are non selective, if associated with the trait- resistance/tolerance will come into the picture along with the resistance.

DR shrimp will resist the disease in grow out system, fetches sustainable production, may be low compare to that of SPF shrimp. In India un avoidable disease manifestation in grow out system is due to haphazard development of grow-out sector without proper drainage, compelling the reuseage of discharged water. Though SPF shrimp yields more production, but susceptible to disease manifestation, leading to unpredictable production. DR shrimp ensures sustainable production, most suitable to Indian conditions to run the industry on a sustainable level. This concept paper was presented in Indo-Singapore Joint Workshop on Frontiers in Aquaculture and Marine Biotechnology held in 2004. Subsequently a microsatellite DNA marker was developed for identifying disease-resistant population of *Penaeus monodon* which will help to separate disease resistant broodstock from those susceptible to WSSV (Mukherjee and Mandal 2009).

3. Resistance/ Tolerance for disease

3.1 Existence of resistance/ tolerance

Shrimp population when subjected to the pathogen in captivity/ culture system some or few animals survive (competent) and the majority of the animals dies with infection (Wang et al., 1998). Competent animals have some sort of internal mechanism to fight against the pathogen and survive. This type of internal mechanism is called resistance/tolerance. In shrimp resistance/tolerance is observed both at species level and individual level within the species. As per the information from hatcheries, most of the wild population of *P. monodon* is carriers of White Spot Syndrome Virus (WSSV) and Monodon Baculo Virus (MBV) and few individuals of the same population are disease free from the same location. Even in a culture's system when stock of monoculture is infected with WSSV some of the population will survive. This is called resistance at the individual level. When wild populations of different species of one location are tested for particular pathogen some are free of that pathogen and some are carriers of that pathogen (Wang et al., 1998). In Taiwan WSBV infection was detected at three stages (Wang et al.,1998) i.e. 1. By external symptoms, 2. PCR first stage amplication (14476p product) and 3. PCR second stage amplication (p416p PCR product). All cultured shrimp (*Penaeus monodon, P. japonicus, P. pencilliatus* and *Metapenaeus ensis*) were positive at all three stages; of the wild shrimps, *Trachypenaeus curvirostris* and *M. ensis* were free at all three stages whereas *P. semisulcatus* was positive only at PCR second stage amplication; and all wild crabs and lobsters are free at three stages. This is indicating the existence of resistance/tolerance at species level. In Poly culture experiments also when disease outbreaks some species will escape from the attack and others will be succumbed. This is indicating the resistance/tolerance at species level. *P. semisulcatus* is resistant to WSSV compare to *P. monodon* (Maheswarudu and Josileen Jose, 2008). *P. stylirostris* is more tolerant to Taura Syndrome Virus than *P. vannamei* (CTSA Publication, 121).

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

An experiment on polyculture with three Indian cultivable Penaeids, namely black tiger shrimp *P. monodon*, green tiger shrimp *P. semisulcatus* and Indian white shrimp *F. indicus* was conducted in a small pond of 0.08 ha area at marine fish farm, Regional Centre of CMFRI, Mandapam. Stocking density was 6.25 post larvae /m^2 and stocking ratio was 25:20:5 for *P. semisulcatus*, *P. monodon*, and *F. indicus*, respectively. Seed (PL_{23}) of green tiger shrimp and tiger shrimp were produced in the backyard hatchery of Regional Centre and seed of Indian white shrimp were collected from wild, nearby Lagoon. Shrimps were fed with commercial pelleted feed No. 1- 4 which were gradually increased in quantity and size of the pellets as the shrimp grew in size. After 70 days as *P. monodon* was infected with WSSV harvest was done. Production was 301 kg/ha and FCR was 1.33. At the harvest survival was 69.3%, 32.2% and 36.7%

for *P. semisulcatusv, P. monodon* and *F. indicus*, respectively (Maheswarudu and Josileen Jose, 2008). Harvested shrimps were segregated species wise and each individual was observed for infection by clinical signs. Only animals of *P. monodon* were infected with WSSV but not other two species. This experiment is indicating that *P. semisulcatus* and *F. indicus* have resistant/tolerant capacity to WSSV compare to *P. monodon*. Wang et al. (1998) found higher mortality rates in experimentally injected (WSBV) shrimps than the respective control groups 18 days post infection, indicating the different mortalities among species tested (*Exopalaemon orientalis, Trachypenaeus curvirostris, Metapenaeus ensis, Macrobrachium sp.* and *Procambarus clarkii*). However, Rajendran et al. (1998) reported 100% cumulative mortality in experimentally infected (WSSV) shrimp (*Penaeus monodon, P. indicus, P. semisulcatus, Metapenaeus monoceros* and *M. dobsoni*) within 5-7 days in injected shrimp and 7-9 days in shrimp fed with infected tissue.

3.3 Experimental evidence for existence of resistance/tolerance at the individual level

An experiment on domestication of tiger shrimp *P. monodon* was conducted during December 1998-September 2002. Two females measuring 225 mm.TL. /120 g. wt. and 245 mm.TL. /135 g. wt. were collected from Palk Bay, introduced into the Rematuration System (RMT) and unilaterally ablated. Males were also introduced in equal number. Since mating has not taken place artificial insemination was carried out. Post-larvae (PL_{15}) were produced from these two spawners and reared up to adult in grow-out ponds of 0.32-0.35ha (one at $13/m^2$ density and another at $8/m^2$ density) separately for 150 days under similar environmental conditions. Of these two populations, one (reared at $8/m^2$ density) has shown fast growth (162 mm.Tl./37 g.wt. versus 143 mm.TL./26 g. wt.)) from which 280 superior (growth) animals of both sexes were selected and transferred into 100 t cement rectangular tank (10x5x2 m) and reared for another 6 months. Broodstocks were fed with the pellet diet, blended with vitamin C, Vitamin E, Ultramin, Fish oil and Cod liver oil. Broodstocks were also given prophylactic treatment with Prefuran, OTC and Formalin periodically. In January 1999 viable post larvae (PL_{17}) of 22,029 of F_2 generation were produced and 18,000 PL_{17} were stocked in 0.15 ha grow-out pond. After 30 days F_2 generation population was infected with WSSV. Pond water was treated with 2 ppm $KMNO_4$ and 50% of water exchange was provided after 24 hrs. Oxytetracycline was added to the shrimp feed for one week at a rate of 2 g/kg. Despite all these efforts, the crop was lost. Then the pond was left without any further water management and feeding. After 88 days 107 animals (58 males+49 females) were found survived from this pond; and were reared up to adult (100 g size) in 100 t cement tank as done in the case of F_1 generation (Mandapam Regional Centre of CMFRI, Mandapam Camp).

Broodstock of F_2 generation (Visakhapatnam Regional Centre of CMFRI, Visakhapatnam) segregated into two groups, one group stocked in RMT along with F_2 generation males to facilitate inbreeding, the other group stocked with males that collected from Bay of Bengal off Visakhapatnam to facilitate cross breeding. Totally 910 PL_{14} was produced from an inbreeding experiment in January 2001 and reared up to adult in indoor fiberglass tanks (5 t capacity). In March 2001, a sum of 37,000 PL_{25} was produced from cross breeding experiment. Of these, 35,000 were stocked in two commercial grow out ponds of 0.8 ha area. Commercial seed, which were stocked in other grow out ponds in the same farm, was infected with WSSV after 50 days. Whereas F_3 generation population resisted up to 65 days with 50% survival and attained the size 134 mm.TL. /18 g .wt. The remaining 2,000 PL_{25} of the cross breeding experiment were reared up to adult in indoor fiberglass tanks (5 t capacity) and conducted breeding experiments to produce F_4 generation. All the survived F_3 generation broodstock of inbreeding and crossbreeding groups responded to eyestalk ablation, matured and spawned, but F_4 generation population could not be produced due to other non scientific reasons. F_3 adults that tested for WSSV were positive only at PCR second stage amplication, indicating the WSSV vertical transmission from F_2 adults, but due to resistance/tolerance at individual level these animals survived. During this domestication experiment, it has been observed that few animals (0.6%) of F_2 generation population survived after infection with WSSV and those survived animals matured to produce F_3 generation, and F_3 generation broodstock of inbreeding group as well as the cross breeding group matured and spawned. This experiment clearly indicates the existence of resistance/tolerance for WSSV at individual level in *P. monodon.* Wang et al. (1998) conducted experimental WSBV infection in wild caught shrimp and observed mortality up to 18 days. All moribund animals during the 18 days were positive for PCR first and second stage amplications where as survived animals only after 18 days were positive for PCR second stage amplication-revealing the potentiality of resistance/tolerance to WSBV in survived animals. Recent studies, 13 years subsequent to the present case report, have confirmed that existence of resistance to WSSV in *Penaeus monodon* of both cultured populations (Chakrabarthy et al., 2014) as well as in wild populations (Datta et al., 2013) and Genetic factor

responsible for resistance was successfully identified (Mukherjee and Mandal, 2009; Robinson et al., 2014).

4. Development of Disease Resistant Broodstock

The proposed research programme on Development of Disease Resistant broodstock of *P.monodon* in captivity involves following steps (Fig.1):

Figure 1: Flow chart showing, breeding program for the development of Disease Resistant (WSSV) broodstock of *Penaeus monodon*

BASE POPULATION	**Challenging with WSSV**	***GENERATION-1***	**Challenging with WSSV**	***GENERATION-2***	**Challenging with WSSV**	***GENERATION-3***	**Challenging with WSSV**	***GENERATION-4***	**Challenging with WSSV**	***GENERATION-5***
Group-1		***Family-1***		***Family-1***		***Family-1***		***Family-1***		***Family-1***
Group-2		***Family-2***		***Family-2***		***Family-2***		***Family-2***		***Family-2***
Group-3		***Family-3***		***Family-3***		***Family-3***		***Family-3***		***Family-3***
Group-4		***Family-4***		***Family-4***		***Family-4***		***Family-4***		***Family-4***
Group-5		***Family-5***		***Family-5***		***Family-5***		***Family-5***		***Family-5***
Group-6		***Family-6***		***Family-6***		***Family-6***		***Family-6***		***Family-6***
Group-7		***Family-7***		***Family-7***		***Family-7***		***Family-7***		***Family-7***
Group-8		***Family-8***		***Family-8***		***Family-8***		***Family-8***		***Family-8***

4.1. Establishment of base population

Base population will be generated by collecting disease free (WSSV) animals of both sexes from different locations of the Indian coast to maintain genetic heterocity. Animals of each location will be maintained separately as a group. Since disease free animals have been selected innate resistance /tolerance/immunity is existed in the selected brood stock. Minimum of 8 groups will be developed from different locations.

4.2. Production of F_1 generation by inbreeding

F_1 generation population of each group will be produced by inbreeding. One pair of male and female will be used for producing progeny once only. Progenies of five pairs will be produced for each group and reared as a family by pooling. In the similar way eight families will be developed.

4.3. Challenging with WSSV family wise

In grow-out system normally WSSV will erupt after 30 days from stock i.e. after attains 3-5 g weight. It is advisable to follow the same period and all eight families will be challenged with WSSV virus separately and mortality is recorded day wise and family wise. Survived animals of each family will be reared up to adult. During rearing period growth is monitored sex wise periodically. After attaining 100 g size (one year) FCR will be recorded for each family. A control group for each family will be run par allay and immune system will be studied in survived animals through haemolymph parameters like total haemocyte count (THC) and Phenoloxidase content in comparison with those of the control group.

a. Haemolymph parameters

Decapods contain three types of haemocytes namely hyaline cells, semi granular cells and large granular cells which are associated with cellular defense. Haemocytes are also associated with proteins like prophenoloxidase which are involved in the encapsulation, melanisation and functions as non-self recognition system. An increase of the total haemocyte count (THC) also provides enhanced immune capability during periods of high activity leading to pathogen resistance in crustaceans. In shrimp there is an innate immunity that originates in the haemocytes and is released during the immune response. Haemocytes play an important role in the cellular immune response, including clotting, self recognition, phagocytosis, melanisation, encapsulation, cytotoxicity, and cell to cell communication. Clotting of haemolymph is a critical mechanism to protect the shrimp from excessive loss of body fluids, as well as to sequester and immobilize invading microorganisms. Haemocyte functions such as phagocytosis, chemo taxis, lysosomal enzyme activities, as well as haemocyte production in the haemopoietic tissue were stimulated by injecting foreign material like bacteria in *P. monodon* (Karin van de Braak and Will PW van der Knaap, 1999).

b. Genetic approach

Information on the molecular and cellular

mechanisms involved in the response of the shrimp immune system to viral attacks is scarce. To develop resistance /tolerance to viral diseases, the genes responsible for the trait –resistance are to be identified. To achieve this, a genetic map for shrimp is needed. Genome mapping is required for establishment of quantitative trait loci (QTL) that responsible for resistance. QTL for resistance is identified by comparing the genome mapping of surviving animals against that of controlled animals. Alcivar-Warren, et al. (1997) conducted experiments on off springs of four crosses (I, II, III, and IV) of *Penaeus vannamei* from known high- and low-growth families to examine the genetic component involved in disease resistance or susceptibility by challenging with infectious hypodermal and hematopoetic necrosis virus (IHHNV) and Baculovirus penaei (BP). On the basis of prevalence of infection and mortality rates, he has drawn a conclusion that the susceptibility to BP is governed by the genetic background of the parental crosses. This proposal was made in 2004, but subsequently QTL for WSSV in *P. monodon* was successfully identified (Robinson et al., 2014), which will help to evaluate the QTL status at each generation of Disease Resistant broodstock in domestication programme.

4.4. Production of F_2 generation

F_2 generation from each family will be produced by random inter crossing between eight families of F_1 generation by following the breeding strategies adopted in production of F_1 generation families. After 30 days all families will be challenged with the virus and survival will be recorded. Based on the survival estimation of heritability will be conducted for resistance transmission. If resistance is a trait and gives positive correlation with the advancement of generations, it is a boost to the shrimp culture industry to combat the WSSV havoc.

4.5. Continuation of the programme up to F_5 generation

This programme will be followed up to the advancement of the F_5 generation and all parameters such as growth, survival, FCR will be recorded at every generation family wise.

4.6. Evaluation of Disease Resistant stock for commercial application

At every generation part of surviving populations of different families (WSSV resistant) will be evaluated in grow out system to record the performance. It is assumed that by advancing to F_5 generation seed that produced from resistant brood stock will perform better against the WSSV in grow-out to give sustainable production.

Conclusion

SFP broodstock with one or two economic traits will perform high in the hatchery to fetch more profits for hatchery sector. A seed that produced from the SPF broodstock will perform well in grow-out system and production will be enhanced with fast growth and low FCR. But horizontal transmission has to be checked by treatment of intake water and recirculation of discharged water in controlled conditions which is an expensive effort. Where as a seed that produced from DR broodstock will resist the WSSV and give sustainable production may be low to that of SPF broodstock. In India grow out sector was spread haphazardly without proper drainage system. Since the arrest of horizontal transmission is expensive and the utmost care has to be paid for development of DR brood stock. In USA High Health Aquaculture Inc. (HHA) is the main supplier of SPF broodstock to the shrimp farming industry, which has supplied Taura Virus Resistant stock of *P. vannamei* for the last three years, causing a 50% increase in total annual production.

Research Highlights

1. The present case report reveals that possibility is there to development of captive Disease Resistant broodstock of black tiger shrimp *P. monodon*.

2. The present case report reveals also the existence of resistance/ tolerance to WSSV in *P. monodon.* About 13 years subsequent to this case report, studies conducted on this aspect also confirmed the existence of resistance/tolerance to WSSV in *Penaeus monodon*.

3. Pond reared *P. monodon* for 150 days, F_1 generation at $8/m^2$ density and F_2 generation at $10/m^2$ density, and subsequent rearing for 6 months in 100 t cement tank at density $3/m^3$ responded to eyestock ablation, matured, spawned and yielded viable post larvae to produce subsequent generation, suggesting to adopt pond culture as well as tank culture for domestication and brood stock development of *P. monodon*.

4. F_3 generation that was reared from the PL to the adult of 100 g size for one year in tank condition, avoiding the initial pond rearing for 5 months as done in the case of F_1 and F_2 generations, reveals that the domestication programme can be carried out exclusively in tank conditions at stocking density $180/m^3$ and $400/m^3$.

5. F_2 generation broodstock those survived from WSSV infection matured, spawned and yielded viable postlarvae to produce F_3 generation, and F_3 generation broodstock matured and spawned,

revealing the possibility for development of disease resistant broodstock for WSSV of *P. monodon* by leading the domestication programme further successive generations, as suggested in the present study.

Limitations

This case report was based on the study done during 1998-2002, which was presented in Indo-Singapore Joint Workshop on Frontiers in Aquaculture and Marine Biotechnology in 2004, highlighting the importance of the development of Disease Resistant broodstock of tiger shrimp. But this concept was not prioritised believing that existence of resistance/ tolerance for WSSV in shrimp is not a validated concept. However, recent studies confirmed the existence of resistance for WSSV in *P. monodon*, both in cultured population and wild population in the sea, promoting this concept to develop Disease Resistant broodstock. Identification of marker for disease resistance, which was developed recently will aid to successfully conduct this proposed programme by evaluating the disease resistance status at each generation and estimating the heritability of disease resistance to successive generations.

Recommendations

1. Many countries initiated domestication and development of captive broodstock of *P. monodon*, but still shrimp culture industry is waiting for viable captive broodstock. Major reasons for failure of domestication programme are 1. Non- response of females and males for inducing breeding during advancement of successive generations, 2. Horizontal transmission of disease due to failure of Biosecurity, 3. Disturbed commitment and dedication of personnel involved with domestication programme.

2. In domestication programme females of shrimp can be induced to breed for a prolonged time by feeding littoral oligochaete *Pontodrilus bermudensis* (Maheswarudu et al., 1996; Radhakrishnan et al., 2000;Vineetha and Maheswarudu, 2013; Maheswarudu and Vineetha, 2013). In the similar way males of shrimp can be induced to produce a spermatophore and to enhance subsequent mating success by applying testosterone hormone usage (Maheswarudu et al., 2015).

3. Horizontal transmission of disease arises in the development of Specific Pathogen Free broodstock and in the performance of SPF broodstock in grow-out sector. Though domesticated SPF broodstock of *P. vannamei* introduced in India, but it is encountering the horizontal transmission of disease in grow-out sector. To avoid horizontal transmission of disease in grow-out sector alternative is to opt for disease resistant brood stock.

4. Domestication and development of captive broodstock of shrimp is a long term goal and only government organizations initiated the domestication programmes due to uncertainty of success of the programme. In government organization's career advancement involves production of a number of publications in prescribed time, which is the limiting factor for the personnel involved with domestication programme, and this issue can be addressed by giving special incentives to the personnel involved with domestication programme.

Funding and Policy Aspects

1. Domestication and development of captive broodstock of shrimp is a long term goal to achieve and it involves long term and after development of captive broodstock different families have to be maintained continuously to maintain genetic heterocity. A separate institute or a separate unit in the organisation have to form exclusively for development of domesticated broodstock. Special allocations of funding have to be made to run the program as preplaned because it cannot be delayed for lack of adequate funds.

2. Domestication programme involves continuous monitoring and only committed and dedicated personnel can discharge the duties. Special incentives have to provide to encourage/ reward their contribution. Isolated places to be selected for domestication programmes to avoid disturbance by other environmental factors as well as to avoid disturbance to working personnel, leading to the success of the programme.

Author's Contribution and Competing Interests

Experiment on domestication of tiger shrimp *P. monodon* was initiated in 1998 by the direction of Dr. K. Gopakumar, Former DDG (Fisheries), ICAR, and Dr. M. Devaraj, Former Director of CMFRI. This work was carried out at Mandapam Regional centre of CMFRI during 1998-2000 and at Visakhapatnam Regional Centre of CMFRI during 2000-2002. This work was designed and carried out by the corresponding author GM. The contributing authors JJ, SM and MRA were associated with this work at Mandapam Regional Centre of CMFRI during 1998-2000 and contributing author**s** UR and CKS were associated with this work at Visakhapatnam Regional Centre of CMFRI during 2000-2002. The corresponding author GM and the contributing author UR

prepared the manuscript. The corresponding author GM has revised the manuscript.

The authors are not competing for financial interest.

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