CHARACTERIZATION OF DIFFERENT STOCKS OF *MACROBRACHIUM ROSENBERGII* AND DEVELOPMENT OF GENETICALLY IMPROVED STRAIN THROUGH SELECTIVE BREEDING

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DEPARMENT OF AQUACULTURE

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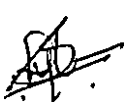
PANANGAD

KOCHI

Dedicated to
my Master & family

DECLARATION

I hereby declare that this thesis entitled "**Characterization of different stocks of *Macrobrachium rosenbergii* and development of genetically improved strain through selective breeding**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, or other title of any other university or society.



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CERTIFICATE

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LIST OF ABBREVIATIONS

AP	Andhra Pradesh
OR	Orissa
KR	Kerala
APOR	Hybrids of Andhra Pradesh male and Orissa female
APKR	Hybrids of Andhra Pradesh male and Kerala female
ORAP	Hybrids of Orissa male and Andhra Pradesh female
KRAP	Hybrids of Kerala male and Andhra Pradesh female
ORKR	Hybrids of Orissa male and Kerala female
KROR	Hybrids of Kerala male and Orissa female
KAK	Population from Achankoil River
KCH	Population from Chalakkudy River
KVA	Populations from Valapattanam River (KKA or Kannur)
KM	Populations from Murinjapuzha

Introduction

1. INTRODUCTION

The giant freshwater prawn (*Macrobrachium rosenbergii*) (de Man, 1879) is the largest Palaemonid in the world (Wowor and Ng, 2007) and is one of the prime species of crustaceans being widely fished and cultured in ponds and rice fields. The natural distribution of *M. rosenbergii* extends from Pakistan in the west to southern Vietnam in the east, across south east Asia, south to northern Australia, Papua New Guinea and some Pacific and Indian Ocean islands, (Mather and Bruyn, 2003). Later broodstocks from Hawaii and south east Asian countries were introduced into many regions, where the species was not indigenous (New, 2000). The modern aquaculture of this species began in early 1960's through the work of Ling (Ling and Merican, 1961; Ling and Costello, 1979). Takuji Fugimura and his team successfully developed larval rearing technique to commercialize the culture of prawn (New, 2000). Today it is widely cultured all over the world and by 2005 production had reached 205,033 tonnes with net value of US$ 896,263,000 (FAO, 2007). Aquaculture production of *M. rosenbergii* in India is 12,806 tonnes in 2008. *M. rosenbergii* is the largest species of the genus, forming the mainstay of freshwater prawn farming in India. It is a suitable species for culture in inlands as well as low lying saline water bodies. It occurs in both coasts of India and is abundant in Bengal and Orissa. It is also reported from rivers of Kerala, Hoogly-Matlaha estuary, Godavary River and Kakkinada Bay.

Information on the genetic structure and variation among cultured species are essential for genetic approaches to optimize conservation strategies and breeding programmes for improving stocks. According to Haig (1998), the greatest contribution that conservation geneticists can make to the assessment of the viability of populations is to separate the component of genetic diversity within the populations from that exists between them.

Different methods can be used to assess the genetic variations of an individual or a population. Traditionally in classical systematics, assessment of

genetic variations is done by examining external morphological traits. But the main drawback of this method is that the morphological traits can often be highly sealed and the expressions can be modified by the environment, hiding the true patterns of relationship. This is because the genotype is fixed at fertilization and consequently cannot be influenced directly by the environment (Nguyen, *et al.*, 2006). Hence special breeding programmes and experimental designs are needed to differentiate genotypic variations from phenotypic variations. In this context taxonomic classification based only on traditional morphometric analysis would not provide correct information. Moreover, the available keys are not adequate to identify larval and post larval stages of prawns. This warrants the use of more reliable and easy identification techniques, which would help us to study the genetic variations of wild populations as well as brood stock selection.

In this regard, biologists have required more fundamental markers to assess genetic relationships. Development of DNA based markers have had revolutionary impact on animal genetics and taxonomical classification by which it is now possible to assess more precisely the genetic variations in the entire genome. These techniques have become a major tool for systematic ichthyologists and may also be useful to fishery biologists for ratification of taxonomic problems at species and population levels (Chow, 1993). Potential sources of direct genetic markers of differentiation include chromosome morphology, protein variants, whole DNA fragments and DNA sequences (Nguyen, *et al.*, 2006). There has been dramatic development of molecular genetics ever since the first widespread use of allozymes in the 1970s. All organisms are subject to mutation due to normal cellular operation or interaction with the environment leading to genetic variation. Through long evolutionary accumulation, many different instances of each type of mutation exist in all organisms and the number and degree of various type of mutation would influence the level of genetic variation within a species (Liu and Cordes, 2004). Presently several markers are used in aquaculture genetics to explore

the genetic diversity of population. Earlier allozyme markers, mtDNA markers Restriction Fragment Length Polymorphism (RFLP), and Randomly Amplified Polymorphic DNA (RAPD), have been used for assessing polymorphism, while markers like Amplified Fragment Length Polymorphism (AFLP), Microsatellite, Single Nucleotide Polymorphism (SNP) and Expressed Sequence Tag (EST) are the recently used ones. According to the gene of known or unknown function, molecular markers are classified into two categories such as Type I and Type II respectively. Type I markers are used to study genetic linkage, quantitative trait loci (QTL) mapping, comparative genomics and candidate gene identification. Type II markers are considered to be non- coding and have been found to have wide spread use in population genetic studies that can be used for the characterization of genetic diversity and divergence within and among populations (Botstein *et al.*, 1980). Much progress has been made recently in the development of various DNA markers in aquaculture species including fish, shrimps and oysters. Parentage and variability assessment have found great utility in aquaculture and the key component in the near future could be QTL mapping. This is because aquaculture genomics generally focus on performance and production traits which are unique to individual organisms. Fishes have some of the most complex mating systems known in the animal kingdom. Effective methods of traceability are required for basic research, aquaculture operations and trade of aquaculture products. With the introduction of powerful markers and an emerging mathematical framework to calculate parentage, it is now possible to analyze genetic relatedness and inheritance in aquaculture (Hastein *et al.*, 2001). The development and application of DNA marker technologies already in progress in other areas such as molecular systematics, population genetics, evolutionary biology, molecular ecology, conservation genetics and seafood safety monitoring, will undoubtedly impact the aquaculture industry in unforeseen ways (Liu and Cordes, 2004).

Among the various markers described above, RAPD a type I marker, first developed in 1990, (Welsh and McClelland, 1990; Williams *et al.*, 1990) uses PCR to amplify small anonymous inverted repeats scattered throughout the genome, with identical random primers of 8-10 base pairs length at low annealing temperatures. Thus, the genome can be scanned more randomly to examine genomic variation without previous sequence information with this technique. Low cost of the technique and the requirement of only nanograms of template DNA are the advantages of RAPD in population and other genetic studies. (Callejas and Ochando, 2001). RAPD primers have been used for species identification (Partis and Wells, 1996) and analysis of genetic diversity in fishes (Wolfus *et al.*, 1997; Hirschfeld *et al.*, 1999; Yue *et al.*, 2002). Since RAPD markers are dominant markers, it is not possible to distinguish dominant homozygous from heterozygous individuals by using this technique. However better technical simplicity and applicability of this marker makes it more preferable than any other markers in stock identification. The RAPD procedure is adopted in this work to evaluate the genetic variation between the wild stocks of *M. rosenbergii* from different regions of India, because it is a simple and does not require any prior information about DNA sequence for primer development.

Like RAPD, microsatellite is another popular molecular marker, which has been used extensively in fisheries research including studies of genome mapping, parentage, kinships and stock structure. Microsatellites consist of multiple copies of tandemly arranged simple sequence repeats that range from 1 to 6 base pairs (Tautz, 1989; Litt and Luty, 1989). Microsatellite polymorphism is based on size differences due to varying numbers of repeat units contained by alleles at a given locus. It is a co-dominant marker with high polymorphism and relative ease of scoring and so it is of large interest for many genetic studies. For genome mapping and marker assisted selection (MAS) it is preferable to use type I microsatellites.

In many countries particularly in south east Asia a rapid decline of wild stocks of *M.rosenbergii* have been reported due to water pollution and habitat loss (New, 2000). Wild stocks can provide an immediate solution for genetic deterioration in cultured stocks and hence it is essential to take steps to conserve these stocks. Documentation of patterns of genetic diversity of wild stock is necessary to identify each stock and prioritize the conservation strategies. Wild populations of *M. rosenbergii* inhabiting the rivers in different geographical areas of India show wide variation in morphological traits and economic characters like growth, disease resistance, meat quality, pigmentation, larval rearing period etc. But very little is known about the genetic diversity of wild stocks. The present work contemplates to study the genetic diversity of wild populations of *M. rosenbergii* collected from different parts of India through morphometric analysis and molecular marker studies. Hence an attempt is made to find out whether the prawns from different areas belong to the same strain or to different strains. Since a large number of DNA techniques and methodologies are available presently for assessing genetic variations of individuals and stocks acquired during the evolution process, the selection of appropriate technology should be done carefully according to the purpose of the study, expense and the facilities available. But care should be paid to choose the most powerful marker within the limitations. Limitations of one marker can be overcome by the use of a combination of markers. Hence RAPD markers would be a suitable technique as it is very simple and provides a rapid solution as explained above. However RAPD marker being quite sensitive to PCR conditions, microsatellite is a more appropriate technique to obtain more precise results. These markers are useful tools to estimate genetic relationship between populations within a species. Hence in this study, combinations of markers such as RAPD and microsatellites have been selected as DNA markers for analyzing the genetic variation among different stocks of *M.rosenbergii*.

It is essential to improve aquaculture production for meeting the future demands of exponentially increasing human population. Genetic improvements of wild stocks have produced high yielding plants and animals. Similarly there is immense scope for genetic improvement of fish for increasing aquaculture productivity. Genetic selection is one of the important methods for genetic improvement that can yield dramatic results. Selective breeding programme exploits heritable genetic variations by selecting superiorly performing individuals from a population for breeding. Selective breeding in aquaculture species is very successful compared to terrestrial animals since there is an average genetic gain of 10 to 20 percent per generation in these aquatic animals (Ponzoni, 2005). This gain could be achieved by selecting genetically superior animals based on their performance. Earlier, selection was done based only on the observable phenotypic characters to infer their genetic makeup without knowing which genes are actually being selected. Most of the economically important traits which are considered in selection programmes are quantitative that are controlled by more than one gene and environmental factors. Hence the effects of underlying genes to the observable phenotypes are very little. But recent advances in molecular genetics have opened possibilities for direct selection on genotype or on linkage association between markers and Quantitative Trait Loci (QTL). The giant freshwater prawn, *M.rosenbergii* is a promising species for aquaculture and different stocks show a wide variation of important economic traits. These variations of traits make it a suitable candidate for genetic selection and improvement helps to overcome the limiting factors for culture. Although *M. rosenbergii* is considered to be a hardy species, the farming of giant freshwater prawn presents several constraints chiefly related to diseases. The domesticated population that is being continuously farmed for several generations is prone to fatal diseases like White Tail Disease, Balloon Disease etc unlike the wild stocks, which appear to be more hardy and disease resistant.

High levels of inbreeding due to sourcing broodstock directly from grow-out ponds and selection of breeders based on their readiness to spawn that often involves early maturing, small females are the reasons for slow growth rate in farmed stocks of prawns (Mather and de Bruyn, 2003). Natural populations of *M. rosenbergii* inhabiting the rivers in different geographical areas of India show wide variation in morphological traits and economic characters. Recent studies have recognized two distinct forms of *M. rosenbergii,* an 'eastern' and a 'western' forms. The production of hybrids between different stocks of *M. rosenbergii* has great economic importance for aquaculture.

The genetic structure of a population is not static and the degree of change depends on the intensity of interventions. Hatchery populations are more prone to changes in their genetic make-up than river populations due to small population size. Inbreeding and genetic drift are other factors responsible for reduction in genetic variation in hatchery population. Loss of genetic variation is considered to be the loss of genetic potential for stock improvement and adaptation to environmental changes. It is therefore essential to monitor any change in the genetic structure of the hatchery population with respect to a base population or wild populations. Selective breeding programme is used to manipulate a population's genes. To accomplish this goal, it is essential to screen the individuals showing good performance of economically important traits based on phenotypic evaluation and genetic characterization. Statistical analysis of the data on population parameters provide an idea whether the population has sufficient phenotypic variation and based on the data the selection of brooders can be done to create a genetically improved population. Heterosis resulting from crosses between strains or between different races or varieties forms an important means of animal and plant improvement (Alam and Islam, 2005). Heterosis and inbreeding depression are particularly strong for fitness-related traits and the amount of heterosis significantly varies among the strain combinations used to make the cross. Thus, the strain combination used for the cross is of fundamental

significance in the utilization of heterosis. Moreover crosses should be done between mildly inbred or between different breeds having genetic variance within them. Therefore, it is important to focus on not only the average amount of heterosis brought by strain combinations but also its variance within each strain combination. Such a strain combination in which the amount of heterosis is large and uniform in every pair is mostly favorable in fish breeding.

Though selective breeding is considered as one of the useful approaches for genetic improvement, it is very expensive and time consuming for obtaining concrete results. Screening strain combinations having genetic variations for superior performance of offspring is the most costly and time-consuming process in hybrid breeding programs. Molecular markers will help to analyse genetic variations of wild stock and develop genetically improved strains for culture through selective breeding of the stocks which show polymorphism.

The present work envisages for studying the genetic diversity of wild populations of *M. rosenbergii* collected from different parts of India through phenotypic characters and molecular marker studies. It also aims to develop a superior strain by combining the desirable characters through selective breeding. This work would help us compare the genetic traits of wild populations of *M. rosenbergii* present in different geographic regions in India and reveal their potential for improvement through selective breeding.

Review of Literature

2. REVIEW OF LITERATURE

The role of aquaculture in increasing food production is significant at this time, since many natural fish resources are over exploited and the yield from capture fisheries is almost stagnating. Crustaceans play a very important role among cultured organisms. Freshwater prawn *Macrobrachium rosenbergii* (de Man, 1875) is one of the commercially important crustaceans, which is a major contributor to global aquaculture both in terms of quantity and value (Wowor and Ng, 2007). Many desirable characters like fast growth rate, disease resistance, compatibility and high market demand make it a preferred candidate for culture. Among 34 *Macrobrachium* species reported from India only seven are commercially important, of which *M. rosenbergii* is the prime one.

2.1 STOCK IDENTIFICATION

Understanding the distribution of genetic diversity in wild and cultured stocks is important for developing sound conservation strategies for preserving wild genetic diversity levels, which are believed to be declining as a result of over-exploitation and habitat degradation (New, 2000). Recognition of unique genetic diversity will also allow the proper choices in breeding programs regarding the selection of genetically diverse brood stock and the maintenance of genetic diversity in cultured stocks (Chand *et al.*, 2005). Hence it is essential to assess the genetic diversity and to maintain the information of the pedigree of the chosen brood stock in implementing a breeding programme.

The giant Freshwater prawn (*Macrobrachium rosenbergii*) is the largest species in the genus and is the most important freshwater prawn species used in culture. *M. rosenbergii* is distributed on East and West coasts of India and different populations exist in various riverine systems (Chondar, 1999).

2.1.1 Morphometric Analysis:

There are many methods used for assessing the genetic variations of different populations and individuals. The traditional method for stock identification approach still employed in classical systematics is by examining external morphological traits to infer divergence in the underlying genes that produce morphological phenotypes. There are a number of problems that can be associated with this approach such as the morphological traits can often be highly conserved and environmental factors can modify expression of underlying genes in diverse ways to hide true patterns of relationship (Nguyen, *et al.*, 2006). As in the case of other organisms, systematic studies of *Macrobrachium* species in the past have mainly been based on morphometrtic analysis. But morphological traits shown to be strongly influenced by the environment in some species of *Macrobrachium* may not be indicative of underlying genetic divergence (Dimmock *et al.*, 2002). In contrast to this wealth of biological information, there have been some problems with the nomenclature of the species (Wower and Ng, 2007). Extensive work had been done in various aspects like fisheries, aquaculture, taxonomy, morphology, development, anatomy, biochemistry and ecology of *M. rosenbergi* (Holthuis, 2000). Johnson (1960 and 1973) recognized two subspecies of *M. rosenbergii* based on morphology. The western subspecies, which occurs on Asian mainland and Malaysia, was identified as *M. rosenbergii schenkeli* (Johnson, 1973) with eastern subspecies being called *M. rosenbergii rosenbergii* (de Man, 1879). Morphometric and allozyme work supports this result (Hedgecock *et al.*. 1979; Lindenfelser, 1984). Buranakanonda (2002) reported that there were three major strains of freshwater prawns in Thailand and of which one is hybrid. Working with *M. australiense,* Dimmock *et al.* (2002) have found that there is much trait variation within and between geographical regions that seems to be related to the environmental factors. Studies with a number of wild specimens revealed the presence of two species of *M. rosenbergii*, easily separated by adult morphological characters. First one is *M.*

rosenbergii (De Man) and second one is *M. dacqueti* widely spread and extensively cultured in America, Asia and Africa (Wowor and Ng, 2007). De Man (1879) and Johnson (1973) reported two forms of *Macrobrachium rosenbergii,* eastern and western, based on external morphology. Recent studies have documented genetic diversity in wild *M. rosenbergii* stocks across the species' extensive natural distribution and have suggested that variation is high and structured spatially among major river drainages (De Bruyn *et al.*, 2004 a,b, 2005; De Bruyn and Mather, 2007).

2.1.2. Molecular Markers

Keys to the emergence of molecular genetics were advances in DNA marker technology. These advances have resulted in a wealth of genetic markers such as allozymes, mtDNA, RFLP, RAPD, AFLP, microsatellites SNP, and ESTs with potentially widespread utility in a variety of aquaculture endeavours (Liu and Cordes, 2004). The usefulness of molecular markers can be measured based on their polymorphic information content (PIC) (Botstein *et al.*, 1980). Various processes such as mutation, selection and genetic drift cause genetic variation within and among individuals, species, and higher order taxonomic groups. Through long evolutionary accumulation, many different types of mutation occur in all species and the intensity of the various types of mutations controls the genetic variation within a species (Liu and Cordes, 2004). DNA marker technology can be applied to reveal these mutations. Large deletions and insertions cause shifts in the sizes of DNA fragments produced upon digestion by restriction enzymes, and can be detected by electrophoresis of the fragments on an agarose gel, while smaller indels require DNA sequencing or more elaborate electrophoretic techniques to determine smaller changes in size. Inversions and rearrangements at restriction sites can be easy to detect because they disrupt the ability of a restriction enzyme to cut DNA at a given site and thus can produce relatively large changes in DNA fragment sizes. Point mutations are more difficult to detect because they do not cause changes in fragment sizes (Liu and Cordes, 2004). DNA markers

can be used to verify pedigrees, screen wild populations to maximise diversity in founder animals and to monitor inbreeding levels in breeding populations and can be used to characterise Quantitative Trait Loci (QTL) and helps in marker assisted selection (Andersson *et al.*, 1994). Molecular markers are classified into two categories: type I are markers associated with genes of known function, while type II markers are associated with anonymous genomic segments (O'Brien, 1991). Type I markers are used in studies of comparative genomics and genome evolution. Due to evolutionary constraints on the genome, many genes and their organization are conserved among species. Comparative genomics deals with the similarity and differences found among genomes. Much time, money, and effort can be saved in developing markers for use in aquaculture genetic studies if genetic information is already available for closely related species (Liu and Cordes, 2004). In general, type II markers such as RAPDs, microsatellites, and AFLPs are considered to be non-coding and therefore selectively neutral. Such markers have found widespread use in population genetic studies, their characterizations of genetic diversity and divergence within and among populations are based on assumptions of Hardy–Weinberg equilibrium and selective neutrality of the markers employed (Brown and Epifanio, 2003). Type II markers also have proven useful in aquaculture genetics for species, strain and hybrid identification, in breeding studies, and more recently as markers linked to QTL.

Several studies had demonstrated the ability to determine parentage, analyze pedigree and identify species using molecular markers (Herbinger *et al.*, 1995; Perez-Enriquez *et al.*, 1999; Sugaya *et al.*, 2002; Jackson *et al.*, 2003; Hara and Sekino, 2003; Chan, 2003; Castro *et al.*, 2004; McDonald *et al.*, 2004; Sekino *et al.*, 2004; Vandeputte *et al.*, 2004).

2.1.2.1. Mitochondrial DNA

Mitochondrial DNA (mtDNA) differs significantly from nuclear DNA in structure and mode of inheritance. mtDNA is a circular molecule containing very little non-coding DNA that undergoes no recombination and is maternally

inherited (Liu and Cordes, 2004). Several characteristics of mt-DNA make it a good choice as molecular marker for population studies (Nguyen *et al.*, 2006). Studies of vertebrate species generally have shown that sequence divergence accumulates more rapidly in mitochondrial than in nuclear DNA (Brown, 1985). This has been attributed to a faster mutation rate in mtDNA that may result from a lack of repair mechanisms during replication (Wilson *et al.*, 1985) and smaller effective population size due to the strictly maternal inheritance of the haploid mitochondrial genome (Birky *et al.*, 1989). Non-coding segments exhibiting elevated levels of variation compared to coding sequences such as the cytochrome b gene (Brown *et al.*, 1993) may be due to reduced functional constraints and relaxed selection pressure. Hence mtDNA markers have been used extensively to investigate stock structure in a variety of fishes (Avise *et al.*, 1986; Graves *et al.*, 1992; Chow *et al.*, 1993; and Heist and Gold, 1999). It can identify large numbers of alleles per loci and are quite popular among aquaculture geneticists in identification of brood stocks. Due to its non-Mendelian mode of inheritance, the mt-DNA molecule must be considered a single locus in genetic investigations (Avise, 1994) and limited number of markers available on the mtDNA molecule positions. Their polymorphic information count (PIC) value is lower than that of other nuclear markers such as RAPD, microsatellites, AFLP, and SNP. Phylogenic and population structures derived from mtDNA data shows variations from nuclear genome due to gender-biased migration (Birky *et al.*, 1989) or introgression (Chow and Kishino, 1995). In addition, mtDNA markers are subject to the same problems that exist for other DNA-based markers, such as back mutation, parallel substitution and rate heterogeneity or mutational hot spots. This method is expensive, particularly for population studies where sample sizes and number of populations may be high (Nguyen *et al.*, 2006).

But in the case of *M. rosenbergii,* few molecular marker based studies have been undertaken till now. Variations in 16s ribosomal RNA and mitochondrial DNA (mt DNA) genes have proven useful for the evaluation of relationship at inter and intra specific level in a number of major crustacean

groups (Sarver *et al.*, 1998; Crandall *et al.*, 1999; Tong *et al.*, 2000; Wetzar, 2001). Mather and Bruyn (2003) studied the genetic diversity of 18 wild stocks of *M.rosenbergii* collected from natural populations of southern Asia to SE Asia and Asia-pacific region using a 472 base pair segment of the 16s rRNA gene and they identified two clades of freshwater prawns on either side of Huxley's line with minimum divergence of 6.2%.

2.1.2.2. Allozyme

The markers that led the revolution in modern genetic diversity analyses were allozymes, which was first developed for analysis of some human metabolic disorders in the early 1960's. Starch gel electrophoresis of allozymes has been the most commonly employed molecular method in fishery genetics (Ryman and Utter, 1987; Hillis *et al.,* 1996). Evolutionary biologists quickly realised that they could be used to investigate the genetic diversity in a wide array of organisms at a much more fundamental level than had been possible previously (Nguyen *et al.*, 2006). Still in widespread use, allozymes were among the earliest markers used in aquaculture genetics (May *et al.*, 1980; Seeb and Seeb, 1986; Johnson *et al.*, 1987; Liu *et al.*, 1992; Morizot *et al.*, 1994). Allozymes are allelic variants of proteins produced by a single gene locus and are used as type I markers. Genetic variations due to changes in DNA can be detected by studying the amino acid sequence of polypeptide chains from different alleles of same enzyme. Isozymes have been widely used as genetic markers to analyse the species and variability among marine animals. Moreover, allozyme variations found in many decapods crustacean species tend to be highly conservative and are not representative of true molecular level of divergence. Previous studies of genetic variation among wild populations of *M.rosenbergii* based on allozyme markers suggested low genetic diversity and failed to detect population differentiation (Sodsuk and Sodsuk, 1998). But Malecha (1977, 1987) and Hedgecock *et al.* (1979) reported three races of *M. rosenbergii* such as eastern, western and Australian, while Lindenfelser (1984) inferred from the analysis of morphometric and

allozyme data that the boundary of eastern and western *M. rosenbergii* is Wallace's line that passes through Indonesia. Allozymes found use in aquaculture for tracking inbreeding, stock identification, and parentage analysis. In a few cases, correlations existed between certain allozyme markers and performance traits (Hallerman *et al.*, 1986; McGoldrick and Hedgecock, 1997). Disadvantages associated with allozymes include heterozygote deficiencies due to null alleles and the amount and quality of tissue samples required. In addition, some changes in DNA sequence are masked at the protein level, reducing the level of detectable variation and some changes in nucleotide sequence do not change the encoded polypeptide and some polypeptide changes do not alter the mobility of the protein in an electrophoretic gel (Ryman and Utter, 1987). Although isozyme systems representing several hundred genetic loci are currently available (Murphy *et al.*, 1996), the relatively modest number of loci usually employed and the low number of alleles exhibited by most loci tend to keep the PIC of these markers fairly modest. Low levels of genetic variation revealed in many allozyme studies of marine fish populations (Siddell *et al.*, 1980; Mork *et al* 1985 and Crawford *et al.*, 1989) prompted a continued search for markers with greater genetic resolution. While new technologies have taken over, allozymes to a large extent provide a relatively inexpensive, quick and sensitive technique for screening genetic diversity (Nguyen *et al.*, 2006). In spite of their strength as co-dominant type I markers, ease of use, and low cost, their use in aquaculture genetics has become limited.

2.1.2.3. Restriction fragment length polymorphism (RFLP)

One of the first technologies to directly target diversity at the DNA level was restriction fragment length polymorphism (RFLP) (Nguyen *et al.*, 2006). RFLP markers (Botstein *et al.*, 1980) were regarded as the first shot in the genome revolution (Dodgson *et al.*, 1997), marking the start of an entirely different era in the biological sciences. In this technique restriction endonucleases enzyme from bacteria, which recognize specific nucleotide

sequences cut DNA in that particular site wherever these sequences are present. This is known as a restriction digestion. The more restriction sites along the sequence, the more places the RE will cut the DNA and hence greater fragments will be produced. By exposing the same piece of DNA from different individuals to the same RE's separately, we can detect any mutations in the restriction sites in individuals from the number and size of fragments produced on a gel that separates DNA fragments by size (Nguyen *et al.*, 2006). Traditionally, fragments were separated using southern blot analysis (Southern, 1976). Thus we can estimate genetic divergence among individuals or populations by comparing the number of DNA fragments produced after restriction digestion. Individuals with identical genotypes will have exactly the same set of fragments for the same piece of DNA.

The major strength of RFLP markers is that they are co dominant markers, because the size difference is often large, scoring is relatively easy. The major disadvantage of RFLP is the relatively low level of polymorphism and sequence information or probes are required, which make it difficult and time-consuming to develop markers in species lacking known molecular information (Liu and Cordes, 2004). This approach is less popular now because RFLP analysis provides information only about the sites at which REs cut and not the larger intervening sequence of DNA and the relatively high cost of the restriction enzymes (Nguyen, *et al.*, 2006).

2.1.2.4. Amplified Fragment Length Polymorphism (AFLP)

AFLP is a PCR-based, multi-locus fingerprinting technique that combines the strengths and overcomes the weaknesses of the RFLP and RAPD markers. AFLP was first employed by Vos *et al.* (1995), AFLP generation begins with the digestion of whole genomic DNA with two enzymes. The unique feature of the technique is the addition of adaptors of known sequence to DNA fragments and used as primer sites for PCR amplification. PCR amplification would result in the production of millions of PCR fragments, for ease of separation by gel electrophoresis. Its primary target of genetic variation

is the same as RFLP, but instead of analyzing one locus at a time, it allows for the analysis of many loci simultaneously (Liu and Cordes, 2004). The power of AFLP analysis is tremendously high for revealing genomic polymorphisms. But it is time-consuming and expensive. Young *et al.* (2001) used the AFLP technique to distinguish different species and their hybrids. AFLP markers also have been used for analysis of meiogynogens and androgens (Young *et al.*, 1996; Felip *et al.*, 2000). Like RAPD, AFLP markers are inherited as dominant markers, (Liu *et al.*, 1998b, 1999c). The major advantages of the AFLP method are its high polymorphism and high reproducibility due to high PCR annealing temperatures. It is more expensive than RAPDs, but because large numbers of loci can be analyzed from a single run, the cost per marker is reduced significantly. Moreover, it does not require any prior molecular information and thus is applicable to any species, including less well-studied fish species. Also like RAPDs, AFLP bands are considered to be bi-allelic and therefore have relatively low PIC scores, but the larger number of loci that can be simultaneously scored greatly increases their utility (Liu and Cordes, 2004). Its major weakness includes the need for special equipment and the use of radioactive labels or special staining techniques such as silver staining.

2.1.2.5. Single nucleotide polymorphism (SNP)

Single nucleotide polymorphism (SNP) used to detect polymorphisms caused by point mutations that give rise to different alleles containing alternative bases at a given nucleotide position within a locus. Such sequence differences due to base substitutions have been well characterized since the beginning of DNA sequencing in 1977, but the ability to genotype SNPs rapidly in large numbers of samples was not possible until the application of gene chip technology in the late 1990s. SNPs are again becoming a focal point in molecular marker development since they are the most abundant polymorphism in any organism, adaptable to automation, and reveal hidden polymorphism not detected with other markers and methods. Though, their PIC is not as high as multi-allele microsatellites, but this shortcoming is

balanced by their great abundance. SNP markers are inherited as co-dominant markers (Liu and Cordes, 2004). Several approaches have been used for SNP discovery including SSCP analysis (Hecker *et al.,* 1999), heteroduplex analysis (Sorrentino *et al.*, 1992) and direct DNA sequencing. DNA sequencing has been the most accurate and most-used approach for SNP discovery. Despite technological advances, SNP genotyping requires specialized equipment.

2.1.2.6. Expressed sequence tags (ESTs)

Expressed sequence tags (ESTs) are single-pass sequences generated from random sequencing of cDNA clones (Adams *et al.*, 1991). The EST approach is an efficient way to identify genes and analyze their expression by means of expression profiling (Franco *et al.,* 1995; Azam *et al.*, 1996; Lee *et al.*, 2000). It offers rapid basic information about gene expression in specific tissue types under specific physiological conditions, or during specific developmental stages (Liu and Cordes, 2004). ESTs are useful for the development of cDNA microarrays that allow analysis of differentially expressed genes to be determined in a systematic way and for gene mapping (Cox *et al.*, 1990; Boguski and Schuler, 1995; Hudson *et al.,* 1995; Schena *et al.*, 1996; Schuler *et al.*, 1996 and Wang *et al.*, 1999). In spite of its popularity in mammalian genome mapping (Yang and Womack, 1998; Amaral *et al.*, 2002; Korwin- Kossakowska *et al.*, 2002 and McCoard *et al.,* 2002), radiation hybrid panels are not yet available for any aquaculture species. Development of radiation hybrid panels from aquaculture species is not expected in the near future, given the fact that physical mapping using BAC libraries can provide even higher resolution and the fact that BAC libraries are already available from several aquaculture species. Therefore, ESTs are useful for mapping in aquaculture species only if polymorphic ESTs are identified (Liu *et al.*, 1999a).

2.1.2.7. Random amplified polymorphic DNA (RAPD)

Advanced DNA techniques are widely used to address problems in many aspects of biology. DNA polymorphisms have been extensively employed as a

means of assessing genetic diversity in aquatic organisms. The development of the random amplified polymorphic DNA technique (RAPD) (Williams *et al*, 1990; Welsh and McClelland, 1990) has provided a useful tool for assessing genetic variability. RAPD consists of PCR amplification of small, inverted repeats scattered throughout the genome using a single, short random primer of arbitrary sequence typically 10 bp long (Williams *et al.* 1990). Thus, the genome can be scanned more randomly than with conventional techniques. Random oligonucleotide primers produce random amplified polymorphic DNA (RAPD) that has been extensively used as molecular markers (Kikuchi *et al.*, 1997; Koh *et al.*, 1999). RAPD technique has been applied successfully in a variety of phylogenetic and population genetic studies (Caetano- Anolles *et al.*, 1991; Bassam *et al.*, 1992; Hardrys *et al.*, 1992; Tingey and del Tufo, 1993; Lynch and Milligan, 1994 and, Borowsky *et al.* 1995). It is an efficient tool to differentiate geographically and genetically isolated populations, and to verify the existence of locally adapted populations within a species, which have arisen due to natural selection or as a result of genetic drift (Fuchs *et al.*, 1998) and for individual and pedigree identification, pathogenic diagnostics, and trait improvement in genetics and breeding programmes (Yoon and Kim, 2001 and Holsinger *et al.*, 2002). RAPD has also been used for phylogenetic studies for species and subspecies identification of fish (Bardakci and Skibinski, 1994; Borowsky *et al.*, 1995; Sultmann *et al.*, 1995 and Partis and Wells, 1996.), gynogenetic fish identification (Chen and Leibenguth, 1995; Corley-Smith *et al.*, 1996) and for gene mapping studies in fish (Postlethwait *et al.*, 1994; Kazianis *et al.*, 1996). RAPD markers have been used for species identification in fishes (Partis and Wells, 1996) , molluscs (Klinbunga *et al.*, 2000 and Crossland *et al.*, 1993) and marine algae (Van Oppen *et al.*, 1996), analysis of population structure in black tiger shrimp (Tassanakajon *et al.*, 1998a), analysis of genetic impact of environmental stressors (Bagley *et al.*, 2001), and analysis of genetic diversity (Wolfus *et al.*, 1997; Hirschfeld *et al.*, 1999; Yue *et al.*, 2002).

The ability to examine genomic variation without previous sequence information (Williams *et al*, 1990), requires of least technology and labour (Caetano-Anolle's *et al.*, 1991; Hadrys *et al.*, 1992 and Black, 1993), the relatively low cost of the technique (Caetano-Anolle's, 1994; Ward and Grewe, 1995; Jones *et al.*, 1997 and Harry *et al.*, 1998) and the requirement of only nano grams of template DNA, are the advantages of RAPD in population and other genetic studies (Callejas and Ochando, 2002). Due to short primers and relatively low annealing temperatures amplification of multiple products is high, with each product representing a different locus. Because most of the nuclear genome in vertebrates is non-coding, it is presumed that most of the amplified loci will be selectively neutral (Liu and Cordes, 2004). The RAPD process typically reveals several polymorphic genetic segments per primer within populations; other segments may appear as monomorphic bands within or across populations (Hardrys *et al.*, 1992). Genetic variation and divergence within and between the taxa of interest are assessed by the presence or absence of each product, which is dictated by changes in the DNA sequence at each locus. The degree of variability observed for many primers will make this technique for individual identification, pedigree analysis, strain identification, and phylogenetic analysis. The random amplified polymorphic DNA (RAPD) technique has proved useful in resolving taxonomic problems, especially in freshwater fishes (Bardakci and Skibinski, 1994; Borowsky *et al.*, 1995).

A major drawback of RAPD markers in population genetic studies is that they are dominant markers (Bardakci, 2001), which fails to distinguish between heterozygous and homozygous individuals possessing a specific allele. In heterozygotes, differences may appear only as differences in band intensity, but scoring of band intensity is very difficult. Hence information on the parental origin of alleles may not get from RAPD markers compared to others. Because of their short length, RAPD markers may produce some defective amplification products, and careful control of DNA quality and amplification conditions is necessary to ensure reproducible banding patterns (Carlson *et al.* 1991; Riedy *et al.* 1992). In addition, the presence of paralogous PCR product (different

DNA regions which have the same lengths and thus appear to be a single locus) limits the use of this marker. Finally, RAPD markers are subject to low reproducibility due to the low annealing temperature used in the PCR amplification. These difficulties have limited the application of this marker in fisheries science (Wirgin and Waldman, 1994).

Despite the disadvantages of dominance and lower reproducibility due to low stringent PCR with RAPD, it has been used for detection of genetic variation in various fish species (Dinesh *et al.*, 1993; Johnson *et al.*, 1994; Foo *et al.*, 1995; Bielawski and Pumo, 1997; Caccone *et al.*, 1997; Cunningham and Mo, 1997). RAPD method uncovered 31 reliable polymorphisms in Atlantic Coast striped bass with five primers selected for population analysis (Bielawski and Pumo, 1997). Callejas and Ochando (1998) successfully used RAPD markers for identification of Spanish barbell species. Thus, RAPD has been used in population studies in fisheries, and can be used efficiently for geographic analysis of populations with differential degrees of geographic isolation. Liu *et al.* (1998a) found that RAPD marker is an efficient tool for making genetic linkage map for improving the breeding efficiency of channel cat fish by marker assisted selection and for tracing commercially important traits. RAPD analysis indicated the presence of three genetically distinct sub regional stocks of four wing flyingfish, *Hirundichthys affinis* located in the eastern Caribbean, southern Netherlands Antilles and Brazil, respectively (Gomes *et al.* 1998). Borowsky and Vidthyanon (2001) conducted studies of genetic variations in four cave and eight surface species of balitorid freshwater fishes from Thailand by using RAPD and reported indices of nucleotide diversity (π) four to five times higher for the surface species than the cave species. The RAPD technique provides a reliable and useful tool for the identification of *Barbus* species (Callenjas and Ochando, 2001). Random amplified polymorphic DNA (RAPD) assay was evaluated for studying genetic relationships and diversities in four species of Indian major carps. Thirty-four arbitrary primers were screened to identify species-specific RAPD markers among *Labeo rohita*, *L. calbasu*, *Catla catla* and *Cirrhinus mrigala* (Barman,

2003). RAPD DNA markers showed lack of major differences between the genetic structures of the broodstock of two Hungarian common carp and reported similar heterozygosity values and allele frequencies (Bartfai, 2003). Random Amplified Polymorphic DNA fingerprinting was used to assess the genetic variability of wild stock from the Amazon River and of three captive stocks that correspond to consecutive generations of neotropical freshwater fish, *Brycon cephalus* (*Amazonian matrincha*) and found a lower genetic variation in farmed fishes than wild fish. They also reported total of 104 fragments that were amplified by the six RAPD primers, 16 loci (15.4%) could be visualized in all the DNA samples of *B. cephalus*, while 88 loci (84.6%) were polymorphic. (Waskol *et* al., 2004). Islam and Alam (2004) reported genetic variation studies with RAPD markers and the lowest genetic variability was found within the hatchery population and similarities with Padma and Jamuna river stocks of carps. RAPD markers generated from 3 PCR primers (#211, #245 and #287) are shown to be alleles present in the genomes of *C. mossambicus* but absent in the genome of *C. fuscus*. Hybrids of *C. fuscus* and *C. mossambicus*, therefore, could possibly be distinguished by the use of these specific molecular markers (Huang, 2005). Identification of masu salmon *Oncorhynchus masou masou* and amago salmon *O. mishikawae* was accomplished using a random amplified polymorphic DNA (RAPD) technique (Yamazaki, 2005).

Random amplified polymorphic DNA (RAPD) analysis was used to amplify the genome of black tiger shrimps (*Penaeus monodon*) to detect DNA markers and assess the utility of the RAPD method for investigating genetic variation in wild *P. monodon* (Garcia and Benzie, 1995; Tassanakajon *et al.*, 1997). RAPD technique was used by Phongdara *et al.* (1999) to identify DNA markers that were specific to the two species of shrimps *Penaeus merguiensis* and *Penaeus indicus*, which are very similar in morphology. These results confirm that the approach of isolating DNA fragments generated by RAPD allowed taxonomic studies in banana shrimps.

Studies with biochemical and DNA markers in fishes by many workers were found to be very effective for assessing the genetic variations of different stocks. Analysis of genetic diversity of two populations of freshwater shrimp *Macrobrachium borellii* with PCR-based technique RAPD revealed the high level diversity scored in this species with RAPD markers in comparison to the low heritability values reported by other authors for decapods, including *M. rosenbergii*, with allozyme markers. These results demonstrate that RAPD polymorphisms can be useful measures of diversity in these taxa (D' Amato and Corach, 1996). See *et al.* (2008) reported the use of RAPD markers to study of genetic diversity and polymorphism of eleven species of *M. rosenbergii* in Malaysia. UPGMA cluster analysis found there is no similarity between the stocks and percentage of polymorphism was also very high. From this study it was found that RAPD based DNA fingerprinting is a useful tool for assessing genetic variability in this species of prawns.

2.1.2.8 Microsatellite

Microsatellites consist of multiple copies of simple sequence having size range from 1 to 6 base pairs representing a unique type of tandemly repeated genomic sequences (Litt and Luty, 1989; Tautz, 1989), which are abundantly distributed across genomes and demonstrate high levels of allele polymorphism. The existence of SSRs in eukaryotic genomes has been known since the 1970s (Bruford *et al.*, 1996). Microsatellites tend to be evenly distributed in the genome on all chromosomes and all regions of the chromosome, both in protein-encoding and non coding DNA (Toth *et al.*, 2000; Liu *et al.*, 2001). Numerous lines of evidence have demonstrated that genomic distribution of simple sequence repeats (SSRs) is non-random (Li *et al.*, 2004). Tautz and Renz (1984) hybridized different microsatellite sequences to genomic DNA from a variety of organisms and reported many types of simple sequences. Abundant in all species studied to date, microsatellites have been estimated to occur as often as once every 10 kb in fishes (Wright, 1993).

SSRs typically span between twenty and a few hundred bases (Beckmann and Weber, 1992). Generally, microsatellites containing a larger number of repeats are more polymorphic, though polymorphism has been observed in microsatellites with as few as five repeats (Karsi *et al.*, 2002). The key feature of SSRs as molecular markers is their hyper mutability and, hence, their hyper variability in species and populations. Microsatellite mutation rates have been reported as high as 10^{-2} to 10^{-5} per locus per generation (Weber and Wong, 1993; Crawford and Cuthbertson, 1996; Ellegren, 2000), which is several orders of magnitude greater than that of regular nonrepetitive DNA (Li, 1997) and the mutation is believed to be caused by polymerase slippage during DNA replication, resulting in differences in the number of repeat units (Levinson and Gutman, 1987; Tautz, 1989). Balloux and Lugon-Moulin (2002) reported alleles with very large differences in repeat numbers, predictive of an infinite allele mode of mutation in some fishes. Each microsatellite locus has to be identified and its flanking region sequenced for the design of PCR primers. For most efficient marker development, microsatellite-enriched genomic DNA libraries are made (Ostrander *et al.*, 1992; Kijas *et al.*, 1994). The fastest and simplest way to detect and characterize a large number of type II microsatellites lies in the construction of small-insert genomic libraries enriched in arrays of tandem repeats (Zane *et al.*, 2002) or can be rapidly developed from Single sequence Repeats isolated previously from closely related species (Bruford *et al.*, 1996). The presence of highly conserved flanking regions has been reported for some microsatellite loci in cetaceans (Schlötterer *et al.,* 1991). SSRs are often highly polymorphic due to variation in the number of repeats (Amos and Pemberton, 1992). They can be simply and rapidly detected by the polymerase chain reaction (PCR) using two unique oligonucleotide primers that flank the microsatellite and hence define the microsatellite locus.

SSR remain relatively stable in bone remnants and dental tissue due to their small size helps the successful application of ancient DNA for molecular analysis (Schneider *et al.*, 2004). In addition, they show a high degree of

allelic variability, and hence uniqueness. Hence microsatellites have become an extremely popular marker type in a wide variety of genetic investigations (Liu and Cordes, 2004).

Over the past decade, microsatellite markers have been used extensively in fisheries research including studies of genome mapping, parentage, kinships, and stock structure (O'Connell and Wright, 1997). For economically important fishes, EST-derived microsatellites have been isolated and used for genetic mapping in zebrafish (Knapik *et al.*, 1998), rainbow trout (Sakamoto *et al.*, 2000), channel catfish (Liu *et al.*, 2001; Waldbieser *et al.*, 2001; Karsi *et al.*, 2002) and Atlantic salmon (Koop and Davidson, 2005). Fishes have some of the most complex sex determination systems known in the animal kingdom (Schartl, 2004). Identification of sex-determining loci is hampered in fish species due to the absence of heteromorphic sex chromosomes (Traut and Winking, 2001), variability of genetic sex determination (Volff and Schartl, 2001) and ability to switch sex depending on the environmental conditions (Baroiller and D'Cotta, 2001). However, applying microsatellites provides a good opportunity to find a sex-determining locus due to specific features in the heterogametic sex such as an obvious reduction in recombination between markers linked to the sex-determination region in male compared to female meioses (Naruse *et al.*, 2000) and the consistently heterozygous status of males for unique alleles in this region (Peichel *et al.*, 2004). Microsatellites are extensively exploited for paternity and relatedness analysis of natural populations, hatchery brood stocks and trade control of fish products, including those from aquaculture (Liu and Cordes, 2004). Fortunately, the development of highly polymorphic genetic markers such as mini- and microsatellites had provided an essential tool for identifying parentage relationships among individuals and obtaining pedigree information in aquaculture selection programs (Ferguson and Danzmann, 1998). Naish and Skibinski (1998) developed five microsatellite markers for *Catla catla* and screened in five river and five hatchery samples from India, Bangladesh and Nepal. McConnell *et al.* (2001) also developed five pairs of microsatellite markers for Catla and

reported the numbers of alleles and heterozygosity observed in a single sample comprising 26 fish from the Halda River. Shikano and Taniguhi (2002) have employed microsatellite marker to estimate the amount of heterosis in various strain combinations of guppy and they found that this marker is a suitable tool for predicting heterosis among hybrids. Alam and Islam (2005) reported seven polymorphic loci in three river populations, (the Halda, Jamuna, and Padma Rivers) and one hatchery population of *Catla catla* by using microsatellite markers. Information about the genetic structure of fishes is essential for fisheries management and for stock improvement programme. Alam and Islam (2005) have successfully used microsatellite marker to exploit the population genetic structure of *C. catla* and Pollack (*Pollachius pollachius*) along European coast (Charrier *et al.*, 2006). Yezbeck and Kalapothakis (2007) isolated five microsatellite loci from the Brazilian piracema species *P. lineatus* and evaluated its usefulness in population genetic studies by determining Hardy-Weinberg equilibrium.

Microsatellites have been used to study several penaeid species such as, *Litopenaeus vannamei* (Wolfus *et al.*, 1997), *Marsupenaeus japonicus* (Moore *et al.*, 1999), *Penaeus stylirostris* (Bierne *et al.*, 2000) and *P. monodon* (Tassanakajon *et al.*, 1998a, 1998b; Xu *et al.*, 1999; Brooker *et al.*, 2000; Pongsomboon *et al.*, 2000). Few studies have been performed in *P. monodon* by using microsatellites (Tassanakajon *et al.*, 1998c; Moore *et al.*, 1999; Brooker *et al.*, 2000). Microsatellite technique was used to study the genetic structure of wild *P. monodon* populations in the Philippines and its association with mangrove status and shrimp culture systems. The genetic diversity of wild populations of *P. monodon* was also compared with that of cultured stocks in an attempt to assess the potential risk of fitness of wild shrimp by the release of cultured shrimp (Xu *et al.*, 2001). Jerry *et al* (2004) reported the use of microsatellite marker for parentage determination in kuruma shrimp (*Marsupenaeus japonicus*). Dong *et al.* (2006) reported the use of a number of microsatellite markers as a realistic and effective alternative to physical tagging in a selection program. It allows the identification of parental effects on

offspring performances of Chinese shrimp *(Fenneropenaeus chinensis)* from early life stages. Zhu *et al.* (2006) isolated five microsatellites for polymorphic loci from Chinese mitten crab (*Eriocheir sinensis*). They reported an average allele number of 16.8 per locus and average expected heterozygosity of 0.88.

Many studies have demonstrated that such markers are ideally suited to study genetic diversity in aquatic organisms (Hedgecock *et al.*, 1996; David and Jarne, 1997; Bierne *et al.*, 1998, 2000; Norris *et al.*, 1999; Primmer *et al.*, 1999; Reilly *et al.*, 1999; Takagi *et al.*, 1999; Taniguchi and Perez-Enriquez, 2000). Microsatellite markers have been shown to be very useful for verifying pedigrees in prawn lines (Moore *et al.*, 1999) and regarded as an invaluable tool in the investigation of genetic diversity and pedigree tracing of hatchery populations (Herbinger *et al.*, 1995; Blouin *et al.*, 1996; O'Reilly *et al.*, 1998 and Perez-Enriquez *et al.*, 1999). Microsatellite marker could be a good choice for the characterization of genetic diversity in both wild and cultivated *M.rosenbergii* due to its reliable informative co dominant nature and ease of exchange of data among different studies (Avis, 1994). The two forms of *M. rosenbergii* have been genetically isolated for a significant evolutionary time frame (de Bruyn *et al.*, 2004a). Chand *et al.* (2005) reported the presence of six polymorphic microsatellite loci in the eastern form of the widespread and commercially important giant freshwater prawn *M. rosenbergii.* They identified number of alleles per locus and observed that heterozygosity per locus ranged from 12 to 18 and from 0.66 to 0.90, respectively in a sample of 29 prawns. Bhassu *et al.* (2005) successfully detected the microsatellite sequences in Malaysian stocks of *M. rosenbergii* for evaluating the genetic diversity. Development of microsatellite primers in freshwater prawn is useful for selective breeding programs and population genetic studies of freshwater prawn. Charoentawee *et al.* (2007) characterized microsatellite loci in a freshwater prawn from enriched genomic library using six biotinylated probes. $(AG)_{10}$, $(TG)_{10}$, $(CAA)_{10}$, $(CAG)_{10}$, $(GAT)_{10}$ and $(TAC)_{10}$. Primers were designed and synthesized for 20 loci. Ten loci were found polymorphic with the number of alleles ranging from five to 17 alleles per locus and the observed

heterozygosity ranging from 0.27 to 0.83 per locus. Poompuang *et al.* (2007) developed eight microsatellite loci from freshwater prawn from Thailand for determining amount of genetic variation among natural and hatchery stocks. High level of genetic variability was observed in natural populations with an average of 4.57 to 20 alleles per locus, and average of observed heterozygosity and expected heterozygosity of 0.57 and 0.75 respectively. All hatchery stocks exhibited levels of genetic diversity similar to natural populations. Bhat *et al.* (2009) isolated and characterized 8 microsatellite loci from a partial genomic library of *M. rosenbergii* using DIG labeled dinucleotides, $(GT)_{15}$ and $(CT)_{15}$ as probes and studied the polymorphism using 24 individuals collected from the wild. All the loci were polymorphic with number of alleles ranging from 3 to 5 and observed heterozygosity 0.50–0.85. Divu *et al.* (2009) have also detected eight polymorphic microsatellites loci by random screening for dinucleotide repeat units within a partial genomic library developed for *M. rosenbergii.*

2.2. INDICES OF LARVAL PERFORMANCE

There is no universally accepted method for determining larval quality, but many criteria are used for evaluating the quality of larvae. Comparison of larval performance between different stocks of *M. rosenbergii* will provide valuable information about the quality of different stocks and their phenotypic variations.

2.2.1 Egg Diameter

In *M. rosenbergii*, fecundity is a function of body weight. Clutch and egg sizes are fundamental life history traits, which directly determine population or individual fitness, especially in aquatic invertebrates where parents do not nourish the young ones (Mashiko, 1992). Intraspecific variations in these reproductive traits have been noted in many species of decapods crustaceans (Efford, 1969; Nishino, 1980) and lower crustaceans (Lonsdale and Levinton, 1985; Belk *et al.*,

1990). Mashiko (1992) conducted egg and clutch size studies in two populations of *M. nipponense* and their hybrids and reported the influence of genetic factor on egg and clutch size.

2.2.2 Hatch Fecundity

Fecundity of berried prawns cannot be directly estimated by using the generally employed techniques (Beganal, 1978). Larval hatch fecundity is the number of larvae released from the egg mass following incubation (Malecha, 1983) and can be calculated by counting the number of larvae hatched divided by the weight of spent female in grams (Brooks, 2001). Fecundity indices are very important tools in hatchery operations for quantifying the requirement of berried prawns for seed production. A number of studies have been conducted for estimating the fecundities of *M. rosenbergii* from different areas (Raman, 1967; Ling, 1969; Jinadasa, 1985; Rao, 1986), but larval hatch fecundity is much more reliable than other fecundity estimations (Brooks, 2001). According to New and Singholka, (1985) and D' Abramo *et al.* (1995), each gram of berried female produces 1000 numbers of larvae but Malecha (1983) and Brooks (2001) reported larval hatch fecundity for *M.rosenbergii* as 400 and 405, respectively. Dinesh and Nair (2003) reported higher LHF for wild caught berries than that of pond reared ones.

2.2.3. Larval Survival

One of the major impediments to culture *M. rosenbergii* has been variable larval survival, which may be due to variation in environment, quality of nutrition (New, 1990) or genetic variations. Bart and Yen (2003) also compared and evaluated the survival rate of larvae of Thai and Vietnam strains of *M.rosenbergii* under uniform environment and observed a survival difference within the first week 92% in Thai group and 55% in Vietnam group, 52% and 16% in fifth week and later in seventh week, it became 33% and 2%.%. Many hatchery managers believe the impact of tank colouration on survival rate and growth of larvae is very high and they claim that dark

coloured tanks increases larval survival rate. Aquacop (1977) obtained best result when the larvae were produced in dark coloured tanks. Bart and Yen (2001) reported that post larval survival up to 36 days was higher for Thai strains of *M. rosenbergii*, 36% compared to Vietnam strain is merely 2%.

2.2.4 Larval Rearing Period

There was no significant difference between the early larval development stages of Thai and Vietnamese stocks of *M. rosenbergii*. However a clear difference in the rate and uniformity of development was evident during later stages (Bart and Yen, 2003). The duration taken for Zoea of *M.rosenbergii* I to VI was 17 days (Uno and Sao, 1969; Kanaujia and Mohanty, 1992) and Kanaujia *et al.* (2003) reported total larval duration of *M.rosenbergii* was 30 to 40 days.

2.2.5 Stress Tolerance

Acute stress tests have been used to distinguish healthy and weak larvae in hatcheries (Maugle, 1988; Tackaert *et al.* 1989; Bauman and Jamandre, 1990; Gomez *et al.* 1991). Tolerance to low salinity and formaldehyde stress were used in *Litopenaeus vannamei* (Samocha *et al.*, 1998), ammonia stress test in *M.rosenbergii* (Cavalli *et al.*, 2000) and temperature tolerance in *M. rosenbergii* by Herrera *et al.* (1998) for analysing larval health.

2.2.5.1 Temperature tolerance

Biologists have been studying thermal tolerance of poikilotherms for more than hundred years considering its versatile significance (Heath, 1884; Hezel and Prosser, 1974; Monica *et al.*, 1996; Beitinger *et al.*, 2000; Fernando *et al.*, 2000; Fernando *et al.*, 2002; Monica and Fernando. 2002). Water temperature has been described as an important factor and fish often compete for favourable temperatures (Magnuson *et al.*, 1979), which evoke multiple effects on organisms (Fry, 1947). A temperature increase beyond the optimal range for any species adversely affects the body functions of that animal

(Crawshaw, 1977) by increasing metabolic rates and subsequent oxygen demand, and assisting proliferation, invasiveness and virulence of bacteria and other pathogens that causes a variety of pathophysiological disturbances in the host (Brett, 1956; Crawshaw, 1977; Reynolds, 1977; Wedemeyer *et al.*, 1999). Temperature affects almost all biochemical and physiological activities of the organism (Magnuson *et al.*, 1979). Elevated temperature can diminish swimming ability in fishes (MacNutt *et al.*, 2004)

Temperature is a direct controlling factor which influences the activity of aquatic organism and, therefore mobile species including the crustaceans show different behavioural responses which include the selection of a thermal habitat and avoidance of lethal temperatures (Reynolds, 1979; Giattina and Garton, 1982). It is well known that small changes in water temperature can have considerable effect on freshwater fishes (Morgan *et al.*, 2001). Metabolic activities of *Macrobrachium sp.* are controlled by temperature and a direct relationship with oxygen consumption and temperature. *M. rosenbergii* can tolerate a wide range of temperature 14 ^{0}C to 35 ^{0}C (New, 1995) and optimum temperature is in the range of 28^{0}C to 32°C and 29 ^{0}C to 31^{0}C, respectively (Sebastian, 1996; Chen and Chen, 2003). Sudden change in temperature induces stress in prawns.

Heat shock (HS) involves the sudden exposure of cells, tissues and organisms to a temperature well above normal and below lethal. The response usually induces the synthesis of one or more heat shock proteins and commonly results in induced thermo tolerance (ITT). These proteins will help to survive, under high temperature conditions (Nover, 1991; Kampinga, 1993; Parsell and Lindquist, 1993). But very little attention has been paid to the stress response and induced tolerance in the area of aquaculture, despite its potential value (Clegg *et al,*. 1998). Comparative approaches that use a single species from different habitats and geographically separated populations offer a better alternative than interspecific comparisons, because species comparisons may be confounded by variables other than habitat differences (Pearson *et al.*,

2002). Thermal tolerances of organisms are usually evaluated by measuring incipient upper and lower lethal temperatures (I_U and I_L) and critical thermal minima and maxima (C_{Max}). The *I* involves abruptly transferring batches of animals acclimated to one temperature into a series of constant test temperatures, either higher or lower. Estimates of the upper and lower temperatures causing 50% mortality for each acclimation temperature are defined as the incipient upper and lower lethal temperatures (IU and IL). 'Zone of thermal tolerance' can be estimated from the data (Brett, 1956). The critical thermal maxima (CTMax) was defined by Cowles and Bogert (1944), modified by Lowe and Vance (1955) and standardized by Hutchison (1961). Cox (1974) defined CTMax as follows: ''these tolerance measurements as the arithmetic mean of the collective thermal points at which locomotory activity becomes disorganized''. This is when the animal loses its ability to escape from conditions that will promptly lead to its death. When heated from a previous acclimation temperature at a constant rate just fast enough to allow deep body temperatures to follow environmental temperatures without a significant time lag (Re *et al.*, 2005). C_M involves subjecting fish acclimated to a specific temperature to rapid temperature changes. The responses recorded in sequence are the temperature at loss of equilibrium (LE), loss of righting response (LR) and onset of spasms (OS). The CTM provides a standard for evaluating the thermal requirements of an organism and is often used to make comparisons among species (Lutterschmidt and Hutchison, 1997). CTMax may occur at different temperatures in different species, but the physiological responses are same across a diversity of taxa (Lutterschmidt and Hutchison, 1997). Hence critical thermal maxima can be used as an excellent index for evaluating the thermal requirements and physiology of aquatic organisms (Becker and Genoway, 1979; Paladino *et al.*, 1980).

A number of studies have already been made on the temperature tolerance and preference mainly of freshwater fish (Coutant, 1977; Becker and Genowa, 1979; Cherry and Cairns, 1982). In some freshwater fish there is a corresponding ontogenetic change in heat tolerance and selected body

temperature, with smaller fish selecting and/or tolerating higher temperatures (McCauley and Read, 1973; Cox, 1974; Reynolds and Casterlin, 1978; McCauley and Huggins, 1979; Jobling, 1994). Cook *et al.* (2006) conducted thermal tolerance of striped bass, *Morone saxatilis* juveniles from Shubenacadie River, Canada, acclimated in fresh water to six temperatures from 5^0 to 30^0C was measured by both the incipient lethal technique (72 h assay), and the critical thermal method (Cm).

The CTM is a preferred method in the field of thermal ecology because of the small number of animals needed and the short time required to complete a test. The use of similar acclimation temperatures and heating rates also facilitates comparisons of data from several studies (Carveth, 2006). Over the last few years, there has been an emerging interest in the effects of temperature on metabolic activity in crustaceans (Whiteley *et al.*, 1996; Whiteley and El Haj, 1997; El Haj and Whiteley, 1997). Critical thermal minima (CTmin) and lower incipient lethal temperature (LILT) were determined for *Penaeus semisulcatus* juveniles at four different acclimation temperatures (14, 20, 26, and 32^0 C) (Kir and Kumlu, 2008).

Nelson and Hooper (1982) conducted thermal tolerance study in freshwater shrimp *Palaemonetes kadiakensis*. They exposed the prawns acclimatized at 20^0C and 28^0C to five different constant temperatures (24^0C, 26^0C, 28^0C, 30^0C and 32^0C). The animals were tested to characterize their upper tolerance limits and to assess their abilities to thermo regulate behaviorally within a horizontal thermal gradient. Herrera *et al.* (1998) observed differences in critical thermal maxima and minima from 37.3^0C to 41.6^0C of *M. rosenbergii* post larvae and juveniles with acclimation temperature ranging from 20^0C to 32^0C. Rahman *et al.* (2004) found that eight day old *M. rosenbergii* larvae tolerated sudden transfer from a temperature of 10^0C to a temperature of 28^0C causes slight mortality while at 39^0C causes almost 100% mortality. Manush *et al.* (2004) reported CT_{max} and CT_{min} 40.73 ± 0.16^0C, 41.06 ± 0.17^0C, 41.96 ± 0.17^0C and 14.9 ± 0.13^0C, 15.4 ± 0.14^0C,

16.98+0.21^{0}C, respectively, for three different acclimation temperatures in four-month-old adult *M. rosenbergii*. Similar results have been previously recorded aquatic organisms (Nover, 1991). A direct relationship was obtained between the critical temperature and the acclimation temperature which increased at intervals of 3–5 1^{0}C (Re *et al.*, 2005).

2.2.5.2 Salinity tolerance

Salinity tolerance was used to quantify the level of inbreeding depression and heterosis because the trait is strongly sensitive to inbreeding and shows a linear decrease with an increase in inbreeding coefficient (Shikano and Taniguchi, 2003). Salinity tolerance is one of the most important fitness-related traits in fish. Shikano *et al.* (1997) revealed that salinity tolerance shows significant heterosis using crosses between the strains. Salinity tolerance is strongly sensitive to inbreeding depression and shows a linear decrease with an increase in inbreeding coefficient using full-sib mating and a closed line of n=10. Therefore, they proposed that the trait of salinity tolerance is one of the most useful indicators to study the amount of heterosis in crosses and inbreeding depression in populations (Shikano and Taniguchi, 2002).

Macrobrachium (Famity Palaemonidae) are considered to have evolved from a marine ancestor (Williams, 1981). Within the genus, there appears to be wide variation in the ability to tolerate saline conditions (Williams, 1980). Salinity is the ecological key factor in the life cycle of *M. rosenbergii,* which inhabits freshwater but the larval and post larval phases are spent in brackish water. Thus, the degree of tolerance towards environmental factors may differ according to phase (Cheng and Chen, 1998a). *M. rosenbergii* larvae and adults are euryhaline to a considerable degree (Goodwin and Hanson, 1975). They can tolerate salinities up to 21 ppt (Fujimura, 1974; Goodwin and Hanson, 1975), 0 to 25 ppt (New, 1995) and 15 ppt (de Bruyn *et al.*, 2004a). Both male and female *M.rosenbergii* adults exhibited strongly hyper-osmotic and hyper-ionic over the range of 0-14% (Cheng, et al., 2003). Rahman *et al.* (2004) reported better survival of previously heat-shocked larvae to hyper salinity

exposures than control larvae of *M. rosenbergii*. Bart and Yen (2003) conducted salinity stress test in two stocks of *M. rosenbergii* larvae and they reported no significant variation in salinity tolerance of larvae. During early development, *Macrobrachium idae* larvae preferred salinities from 5 to 20 ppt. In these salinities all larvae moulted after 24 h; their median tolerance (Subramanian *et al.*, 1980).

2.2.5.3 Ammonia tolerance

Ammonia is the main excretory product of crustaceans (Hochachka and Somero, 1973; Kinne, 1976), and its modes of toxicity and lethal concentrations to a variety of organisms have been documented (Warren, 1962; Campbell, 1973). Concentrations of ammonia in the blood of crustacea range from 2 to 18 mg/litre (Mangum *et al.*, 1976), which is greater than in their habitat (Kinne, 1976) and diffusion of NH_3 is a principal route of excretion. As external NH3 concentrations increase, the rate of diffusion outward from an animal decreases and body load of ammonia exceeds its tolerable level. The toxicity of ammonia to aquatic organisms is generally due to the NH_3 molecule (Ellis, 1937; Spotte, 1970; Hampson, 1976), despite evidence that NH_4^+ adversely affects some physiological functions (Shaw, 1960; Maetz, 1972; Campbell, 1973). Ammonia exists in solution primarily as the NH_4^+ ion and the un-ionized NH_3 molecule, the proportions of which are highly pH-dependent (Whitfield, 1974; Emerson, 1975).

Ammonia and nitrite are the most common pollutants in culture systems and high levels of ammonia and nitrite in water are potential factors triggering stress in organisms (Lewis and Morris, 1986). Short term ammonia toxicity can be used as an evaluation criterion for larval quality. There has been little work done on the sensitivity of crustaceans to ammonia poisoning. Adverse effects of ambient ammonia on some physiological functions have also been reported by Shaw (1960), who found a significant reduction in sodium influx in the crayfish *Astacus pallipes*, and Mangum *et al.* (1976), who reported reduced ammonia excretion rates in the blue crab, *Callinectes sapidus*. The exposure

levels of ammonia in these experiments were high, 18 and 180 mg NH_4^+/litre, respectively, and may have been approached lethal concentrations. Wickins (1976) found that 101 mg ammonia/litre (pH - 7.0) gave an LC_{50} of 24 h for adult *Macrobrachium.* Armstrong *et al.* (1978) reported the LC_{30} values for 144h of ammonia to *Macrobrachium* larvae at pH 6.83, 7.60, and 8.34 were 80, 44, and 14 mg ammonia/ litre respectively. They also found that the 24 h LC_{50} values were 200, 115 and 37 mg ammonia/litre at pH 6.83, 7.60 and 8.34, respectively and the sensitivity to ammonia remained greatest at higher pH values throughout these tests. Chakraborty *et al.* (1999) conducted work to evaluate the effect of water quality parameters on larval rearing of giant freshwater prawn. Cavalli *et al.* (2000) conducted short term ammonia toxicity test for assessing larval quality of freshwater prawn and they found that prawns fed with nutritious diet showed better tolerance. Rahman *et al.* (2004) reported the same survival level for the larvae of *M.rosenbergii* before and after heat shock when challenged with ammonia toxicity. Unlike ammonia, nitrate is found to be less toxic to *M, rosenbergii* larvae. 180 mg/l of nitrate had no effect on early larval stages (I to VIII stage), but high concentration of nitrate (1000 mg/L) affected the growth. But in last larval stages (VIII to post larvae) higher concentration of nitrate affected more seriously on growth rate, metamorphosis and respiration rate (Ismael *et al.,* 2004).

2.2.5.4 Formaldehyde tolerance

Bart and Yen (2003) evaluated the formalin tolerance of two stocks of *M. rosenbergii* larvae and they found no significant variation in formalin tolerance of larvae. Samocha *et al.* (1998) used formalin stress test as an index for larval health in *Litopenaeus vannamei.*

2.3 ECONOMICALLY IMPORTANT TRAITS

2.3.1 Growth

The most important trait to be considered during selection is growth rate. The major problem encountered in the culture of freshwater prawn is its wide range of sizes especially in males. The growth of many prawns is stunted particularly under high stocking density (Karplus *et al.*, 2000). Based on the growth rate, juveniles are categorised into two, jumpers and laggards. Jumpers are fast growing prawns, become blue and orange clawed prawns, which exhibit fifteen times more growth rate than population mode (Ra'anan and Cohen, 1984a, 1985; Karplus and Hulata, 1995), where as the laggards become the small males (Karplus *et al.*, 1986; Karplus *et al.*, 1987). Heterogeneous growth is predominantly found in males, where three morphotypes small male, orange clawed males and blue clawed males exhibited size variations (New, 1995). Size variation is influenced by genetic and environmental components. Heritability of size has been found to be sexually dimorphic trait, in males genetic control of size was found to be zero, while in female heritability was nearly 0.35 Malecha *et al.* (1984). Sun *et al.* (2000) reported a substantial difference in androgenic gland protein and cellular morphology between the morphotypes of *M.rosenbergii*. Ranjeeth and Kurup (2002a) mentioned the possibility that the differential growth in male prawns could have a genetic basis. Zacarias (1986) reported that early hatching larvae are to become early settling PL, which in turn become the larger prawns. But this is not in agreement with the result of Ranjeeth and Kurup (2002b). They have found that first hatched weaker larvae take more time for metamorphosis. Many studies found that the social interactions also play an important role in the control of growth in prawns (Ra'anan, 1982; Ra'anan and Cohen, 1984b; Ra'anan and Cohen, 1985).

2.3.2 Disease Resistance

Phatarpekar *et al.* (2002) reported that the predominant bacterial flora found in hatcheries were Gram- negative bacteria such as *Aeromonas, Alcaligenes* and *Pseudomonas* and *Enterobacteriaceae. Streptococcus* were also abundant on the larvae themselves. Nagarajan and Chandrasekar (2002) reported that white muscles or idiopathic muscle necrosis, ciliate infestations, antenna and tail rot, soft shell and hard shell were some of the common problems encountered in the rearing system of freshwater prawns in India. *Enterococcus spp.* is found to cause muscular necrosis and mass mortality in *M. rosenbergii* (Cheng and Chen, 1998b). The condition for optimal growth of *Enterococcus spp is* pH 7-8, temperature 27-30^{0}C and 0.5-1% NaCl (Cheng and Chen, 1999). Ammonia in water decreases the virulence of this micro organism and decreases the immune resistance of *M. rosenbergii* (Cheng and Chen, 2002).

Studies to understand crustacean defense mechanism will help to identify the stocks having more disease resistance and to overcome the disease problems. Though many workers have tried to study the defense mechanism but the information gathered about the disease resistance of prawns is not enough to reach a conclusion. This may be due to the lack of effective indictors for measuring its immune status (Sritunyalucksana *et al.*, 1999). The primary immune response in crustaceans is non-specific cellular immunity (Anderson, 1992). Decapod crustaceans have three types of haemocytes, each having distinct morphology and physiological function (Johnson *et al.*, 2000). Circulating haemocytes are responsible for cellular defense mechanism, which participate in coagulation, phagocytosis, encapsulation, and cytotoxic mediation (Johansson and Soderhall, 1989; Hose and Martin, 1989; Bache`re *et al.*, 1995). In addition to this, humoral response involves the synthesis of several antibacterial proteins (Bowman and Hultmark, 1987), which plays a major role in crustacean immune response system. The terminal enzyme in the prophenoloxidase (proPO) activation system is activated by many substances

including several microbial polysaccharides (Smith *et al.*, 1984,). Phenoloxidase activity is detectable in the granular cells (GC) and semi granular cells (SGC) of the giant freshwater prawn (Sung *et al.*, 1998). Phenoloxidase stimulates cellular defense mechanism and plays an important role in melanisation.

2.3.3 Meat Quality

Ninan *et al.* (2003) estimated the proximate composition of *M. rosenbergii* and reported a protein content of 19.44%. Sebastian and Ramachandran (2003) had also conducted proximate analysis of freshwater prawn from different areas.

2.4 CAGE CULTURE

Nursery system is an important component of freshwater prawn culture operation. Bigger prawns require shorter culture periods (Apud *et al.*, 1983) and yield better growth and survival than direct stocking (Shigueno, 1970; Honma, 1971). Cages can be used to conduct nursery rearing of PL of *M. rosenbergii* (Sandifer *et al.*, 1983; Wulf, 1982; Smith *et al.*, 1983; Ang and Law, 1996). Paramaswaran *et al.* (1992) found that there was no difference in growth and survival when prawns were stocked at a density of 200, 300 and 400 PL/m^2. Naik *et al.* (2003) conducted nursery rearing of prawns in cages and they reported that the growth rate and survival rate of cultured organisms were inversely proportional to stocking rate. Similar observations were reported by Sandifer and Smith (1975), Wills and Berringan (1977), Singh and Qureshi (1977).

2.5. SELECTIVE BREEDING

Selection and hatchery practices are the prime factors causing reduction in genetic variability of aquaculture populations. For freshwater prawn, common practices of using gravid females to produce larvae may also lead to a

reduction of the effective population size and subsequently to inbreeding. Keeping breeding records and pedigree information of breeders help to prevent the chance of inbreeding in later generations.

Aquaculture production systems in developing countries are based on the use of unimproved species and strains and the availability of genetically more productive stock becomes vital in order to utilize the resources more effectively (Ponzoni, 2006). Many genetic diversity studies reported a reduction in genetic variation in hatchery stocks of fishes and crustaceans such as tiger prawn (Xu *et al.*, 2001), Japanese flounder (Sekino *et al.*, 2002), Nile tilapia (Brummett *et al.*, 2004), channel catfish (Simmons, *et al.*, 2006), and Kuruma prawn (Luan, *et al.*, 2006). A key factor to boost production of cultured species is development of genetically superior brood stocks that are adapted to culture conditions and market characteristics such as improved growth rate, food conversion efficiency, disease resistance, cold tolerance, tolerance to low Oxygen and the ability to use low cost non animal protein diet (Hew and Fletcher, 1997). Genetic selection is by imposing differential reproductive opportunities for individuals of the population for replacing a given population of genotypes, with another having superior phenotypic performance (Jahageerdar, 2003).

Selection is an efficient method in animal breeding programme, which can lead to the long term goals of genetic goals. There have been a number of selection procedures have been used in animal husbandry, but all of them cannot be suitable in aquaculture (Uraiwan, 2002). Doyle and Talbot (1986) developed modified selection method in which the selection can be done on size-specific growth rate, which help to avoids phenotypic variation due to different time of spawning and hatching. Within family selection is another selection procedure, where the selection is based on the deviation of each individual from the mean value of the family to which it belongs (Falconer, 1981). Within family selection was used in selective program to improve growth rate of giant freshwater prawn (*M. rosenbergii*), which has been carried

out at the Aquatic Animal Genetics Research and Development Institute during 1998 to 2000 (Uraiwan *et al.*, 2003). There is another type of selection procedure called combined selection, where the animals were selected based on their individual phenotypic value and the phenotypic value of the family to which they belong.

Though Selective breeding in aquaculture species has been very successful, averaging a genetic gain of 10 to 20 % per generation (Ponzoni *et al.*, 2005) and the selection response for growth rate in aquatic species is very good, 20-35 percent in fish and shellfish, compared to terrestrial animals. But aquaculture is still in its infancy with respect to farming animals and the development of superior brood stocks by selection is still relatively slow. In 1993, less than 1% of world aquaculture production was based on brood stock originating from selective breeding programs (Gjedrem, 1997). However, in recent decades, substantial genetic improvement and increases in production efficiency have been achieved in some farmed fish species such as salmonids and tilapia (Hulata, 2001). During the last decade, efforts have been made to enable the incorporation of molecular genetic information in genetic improvement of both plants and animals. However, the benefits from the use of these technologies will not be fully utilized unless the cost of genotyping is reduced (Dekkers and Hospital, 2002). Majority of seeds are produced from genetically inferior, low performing wild or cultured animals due to genetic erosion as a result of genetic drift or in breeding (Donato, 2008). Detailed study on genetic variations of wild stock based on molecular markers will help to select brooders of high genetic polymorphism to develop genetically improved animals through selective breeding of the stocks to produce offspring's of high heterosis and can develop better strains for culture. It will also help to identify genetically distinct populations, which should be conserved and can be used for hybridization for the development of new strains showing hybrid vigor for the use of culture practices. Hence selection on the basis of genetic variation will help to minimize the deleterious effects of inbreeding, which can result in a reduction in the mean phenotypic value of

fitness traits such as reproductive capacity and physiological efficiency (Falconer and Mackay, 1996) and knowledge of parentage, should be known clearly to ensure mating among unrelated individuals. After getting reliable pedigree information mating can be arranged to minimize inbreeding (Norris *et al.*, 2000). Heterosis resulting from crosses between strains or between different races or varieties forms an important means of animal and plant improvements. Heterosis and inbreeding depression are particularly strong for fitness-related traits of out breeding species. In general, the amount of heterosis significantly differs among the strain combinations used to make the cross (Falconer, 1989). Mating trials carried out between different geographic races showed that there was no reproductive barrier to gene exchange among different races and most crosses were fertile (Sarver *et al.*, 1979; Malecha, 1987). But a recent re-analysis of mating trial data suggested mating between individuals from widely separated geographic regions resulted in reduced larval survival (Mather and Bruyn, 2003). Therefore, it is important to focus on not only the average amount of heterosis brought by strain combinations but also its variance within each strain combination. Such a strain combination in which the amount of heterosis is large and uniform in every pair is mostly favourable in fish breeding. But screening of such strain combinations for superior hybrid production is very costly and time-consuming process in hybrid breeding programs. The amount of heterosis and F1 performance will depend on the phenotypic values of parents and the level of genetic diversity between the strains used for the cross (Shikano and Taniguchi, 2002). In contrast, crosses among mainland Asian wild stocks of *M. rosenbergii* from Thailand and Malysia showed the evidence of hybrid vigor in the same geographical races (Dobkin and Bailey, 1979). Interspecies hybridization has been widely used in the development of aquaculture in some fish species and in crustacean species but without much impact due to the more complicated, and less known reproduction system in crustaceans (Moreno *et al.*, 2000).

Super Tilapia or the programmes of Genetic Improvement of Farmed Tilapia (GIFT) was developed at World Fish Centre through selective breeding

of several strains of Nile Tilapia, which showed faster growth rate and better survival (Anonymous, 2005). This indicates the importance of comparative works on various strains of freshwater prawns, and the potential improvements that could be gained not only from selection but also from examining whether different strains have different rearing requirements, which probably arise from the genetic diversity that would be established using molecular marker analysis. Improved Jayanti -rohu- the first genetically improved fish of India a project on the genetic improvement of rohu, particularly for better growth performance through selective breeding was initiated for the first time in India in 1992 at the Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, in collaboration with the Institute of Aquaculture Research (AKVAFORSK), Norway. After four generations of selection, an average of 17 per cent higher growth per generation was observed in improved rohu (Mahapatra, 2006).

Early work on the improvement of economically important traits in Penaeids was related to domestication and culturing in closed cycles (Aquacop, 1983; Lester, 1983) followed by the study of growth related traits (Lester, 1988; Goswani *et al.*, 1990; Huang *et al.*, 1990; Wyban, 1992). Later the genetic improvement works not only focused on growth related traits but also on disease resistance especially to viral diseases (Argue *et al.*, 2002; Coman *et al.*, 2002; Goyard *et al.*, 2002; Gitterle *et al.*, 2005a, b; Kenway *et al.*, 2006). Artificial selection has been widely used to improve growth performance and disease resistance in several species of penaeids. French scientists successfully developed IHHNV resistant *Penaeus stylirostris* through selective breeding of survivors of specific pathogen infected stocks (Lightner, 1996). Specific Pathogen Resistant (SPR) *Litopenaeus vannamei* that showed improvement in harvest up to 20-40% was also developed at Venezuela through selective breeding of TSV (Taura syndrome virus) resistant domesticated strains (Lightner, 1996). The development and utilization of genetically improved fish is spreading across the world (Dunham, 1997), but it is not in the case of prawns except genetically improved *L. vannamei* (Benzie, 1998). Fjalestad *et*

al. (1999) have found significant differences between both half- and full-sibs for harvest weight and survival of infection with TSV in *L. vannamei*. Selective breeding has also improved growth of the shrimp Marsup*enaeus japonicus* (Hetzel *et al.*, 2000). A genetic improvement programme was conducted in Pacific oysters, *Crassostrea gigas*, in Australia (Ward *et al.*, 2000). The improvement programme tried to combine family and mass selection with molecular genetics. Two generations each of mass selection and family selection showed an improvement of growth rate about eight percent in the first generation from a mass selection. Argue *et al.* (2002) established two *L. vannamei* breeding lines, one selected entirely for growth and the second selected on an index weighted 70% for TSV resistance and 30% for growth. De Donato *et al.* (2005) reported that the gain in growth rate produced in *L. vannamei* by mass selection was 14.5% after 11 generations. Donato *et al.* (2008) investigated the effects of family selection on the growth rates of four genetic lines of *L. vannamei*, the Pacific white shrimp, from Venezuela. The mean growth rates per generation were 0.141 g d^{-1} for the parental generation (P_0), 0.173 g d^{-1} for the F_1 and 0.191 g d^{-1} for the F_2. Survival varied from 83.3% to 94.4%.

In freshwater prawns gravid females from the wild or hatchery stocks are usually used for the production of larvae without assessing genetic variation and selection may also lead to a reduction of the effective population size and subsequent inbreeding. Experimental mating trials were carried out among the three geographical races of *M. rosenbergii* showed there is no evidence for the influence of reproductive barrier for gene flow and most crosses were fertile (Sarver *et al.*, 1979; Malecha, 1987). Cross between Thailand and Malaysia stocks showed hybrid vigor (Dobkin and Bailey, 1979). Rattikansukha (1993) conducted interspecific hybridization of *M. rosenbergii* from different regions by reciprocal cross. Bart and Yen (2001) found that larval survival was much higher for Thailand strain of *M. rosenbergii* compared to Vietnam strain and cross breeding of these two strains improved the survival rate of offspring. Dimmock *et al.* (2002) reported that the marked

trait variations within and in between geographical areas might be due to environmental factors. Different populations of *M.rosenbergii* exist in various riverine systems of India. Large variation has been observed in its body weight, length, disease resistance, growth and survival and other economically important traits, which offer an opportunity for genetic selection and improvement (Jahageerdar, 2003). Uraiwan *et al.* (2002) conducted sib analysis and selection procedure was designed to estimate heritability on growth rate of *M.rosenbergii.* Genetic selection is an important tool to overcome the limiting factors for culture and to improve the production. Graziani *et al.* (2003) reported that both full sib and half sib families of *M. rosenbergii* might be produced by natural as well as artificial insemination techniques. Selective breeding programme of *M.rosenbergii* to improve growth rate by within family selection was carried out by Uraiwan *et al.* (2003).

An alternative approach to improve the productivity of cultured stocks is cross breeding, which helps to exploit heterosis of off springs. But this technique has been trailed only sparingly in aquaculture; examples exist of hybrid crosses with superior performance to pure lines, common carp *Cyprinus carpio* (Bakos and Gorda, 1995; Hulata, 1995). Crossbreeding has been used to transfer favourable traits among strains (Fjalestad, 2005). Crossbreeding can be a relative simple and inexpensive method for improving local strains (Fjalestad, 2005).

2.5.1 Heritability

The genetic gain from selective breeding can be estimated from heritability, which is a key factor to determine the genetic progress after selection. It measures the proportion of contribution of genetic factor in the final expression of a quantitative character (Padhi and Mandal, 2000).

Several characteristics of larvae and postlarvae of *L. vannamei* and *P. stylirostris* had large errors which encompassed the whole range of heritability (0-1) and found that the strong influences of environmental factors on prawn

larval growth (Lester, 1988; Lester and Lawson, 1990). Gjedrem (1992) found that the comparisons of half-sib groups are less affected by environmental influences. Benzie *et al.* (1997) reported the analysis of the heritability for total length and wet weight of 6- and 10-week-old postlarvae of *P. monodon.* Fjalestad *et al.* (1999) reported a genetic gain of 4.4% for harvest weight and 12.4% for survival after a challenge test using a selection index that equally weighted both factors, and also estimated heritability between 0.45 and 0.50 for harvest weight and 0.22 and 0.35 for survival.

Meewan (1991) estimated the heritability of growth among full and half sib families of freshwater prawn, *M. rosenbergii* and the heritability estimate on paternal, maternal and full sib analysis were found to be 0.40. Rattikansukha (1993) conducted interspecific hybridization of *M. rosenbergii* from two rivers by reciprocal cross and the hybrid did not exhibit heterosis. Uraiwan *et al.* (2003) found during the selective breeding of *M.rosenbergii* that there was a significant improvement in length and weight than control and an average heritability for length and weight was 0.38 and 0.22.

Materials and Methods

3. MATERIALS AND METHODS

3.1 GENETIC CHARACTERIZATION

3.1.1 Sample Collection

Freshwater prawn samples were collected from different rivers of India viz: Achankoil (KAK), Chalakudi (KCH), Valapattanam (KVA) and Murinjapuzha (KM) and were pooled together as Kerala (KR), Mahanadi of Orissa (OR) and domesticated hatchery stocks from Andhra Pradesh (AP) for DNA marker studies. Fresh pleopods were collected from each prawn by using sterilized scissor and forceps and were preserved in labelled bottles containing 75% alcohol.

3.1.2 DNA Extraction

Genomic DNA was isolated by modified salt extraction method (Crandall *et al.*, 1999) from alcohol preserved tissues after rinsing four times with Tris buffer having pH 8 by spinning between washes. The procedure used for isolation of DNA from tissue was as follows:

1. The tissue samples were homogenised in a sterile mortar and pestle and minced tissues were transferred to 2 ml vials.
2. 500ml of solution 1 (50 mM Tris HCL, 20 mM EDTA and 2% SDS in ddH_2O; pH-8) was added to the vials.
3. 5 µl of Protinase K (20mg/ml) was added to the vials and incubated at 55^0C in a water bath for 2 hours with intermittent stirring.
4. After incubation, the vials were chilled on ice for 10 minutes.
5. 250 ml of solution 2 (Saturated NaCl: 6M) was added to the vials and inverted several times to mix.
6. The vials were chilled on ice for 5 minutes and centrifuged at 8000 rpm for 15 minutes in a refrigerated centrifuge.

7. 500 µl of clear supernatant was collected carefully in new labelled eppendorf vials.
8. 1.5 µl of RNase having a concentration of 20 µg /ml was added to the vials and incubated at 37 ^{0}C for 15 minutes.
9. 1 ml of cold 100% AR grade ethanol was added to the vials to precipitate the DNA. The vials were kept in -20^{0}C for 2 hours and centrifuged at 11,000rpm for 15 minutes.
10. After removing the supernatant the DNA pellets were rinsed in 500 µl of cold 70% ethanol and centrifuged at 11000 rpm for 5 minutes.
11. The supernatant was removed carefully and the pellet was dried.
12. Extracted DNA was resuspended in 30 µl of DEPC treated D-Nase, R-Nase free water and stored in -20 ^{0}C.

3.1.3 Determination of Quality and Quantity of DNA

The quality of DNA samples isolated were checked electrophoretically by running 5 µl of DNA on 0.7% agarose gel with TAE buffer. The concentrations and purity of DNA samples were checked with spectrometer (Eppendorf Bio photometer Germany) are given in Table 8 and 9. For this 2 µl of stock solution of DNA was taken in cuvette and diluted with 48 µl of double distilled water. Concentration and purity were measured with spectrometer.

3.1.4 Random Amplified Polymorphic DNA (RAPD) Analysis

3.1.4.1 DNA used

Eighty two DNA samples extracted from pleopods of prawns collected from rivers of Kerala, Orissa and Andhra Pradesh were used for RAPD analysis.

3. 1.4.2 Primers used

Commercially available primers from Bio serve Biotechnologies, India were used for this analysis. Five numbers of decanucleotide/ decamer primers (Table: 1) screened from a total of twelve OPA primers (Bio serve Biotechnologies, India) based on the polymorphism, were used to amplify genomic DNA.

Primers were resuspended in sterile DEPC treated DNase and RNase free water to make stock solution of 100 pm/µl concentration. The stock solutions were stored in -20 ^{0}C. Working solutions were prepared from the stock solutions by diluting ten times with sterile water to get a final concentration of 10 pm/µl.

3. 1.4.3 Template DNA

The working solution of DNA was prepared from stock solution of DNA by using double distilled DNase and RNase free DEPC treated water to get a final concentration of 20 ng/µl DNA. 1 µl of working solution of DNA was used for every 25 µl PCR reaction volumes.

3.1.4.4 PCR conditions

Samples were amplified in a total volume of 25 µl containing 20 ng/µl of DNA, 1X Taq buffer (10mM Tris, 15 mM $MgCl_2$,Gelatin), 0.5mM dNTP mix, 10 pmols of each primer, 3U of Taq polymerase and deionised water. Amplification was done in a gradient thermal cycler (MJ Mini Bio-rad) with an initial denaturation of 3 min. at 95^{0}C followed by 39 cycles of denaturation at 94^{0}C for 1 min, suitable annealing temperature for each primer for 1 min and finally an extension step at 72^{0}C for 1.3 min., followed by a final extension of 72^{0}C for 10 min. After amplification the products were stored in -20^{0}C till use.

3.1.4.5 Agarose gel electrophorosis

Horizontal submarine electrophoresis was done using horizontal gel electrophoresis unit (Bangalore Genei, Bangalore). The gel was cast in gel holder placed in gel casting unit fixed with clamps. Comb was placed in the notch of the gel holder. Electrophorosis of PCR products were done on 2% agarose gel prepared in 1X TBE buffer (Tris-base, Boric acid, EDTA and De ionised water). For this 2 g of agarose powder (low EEO) was weighed and transferred to a 250 ml conical flask and added 200ml of 1X TBE buffer. The mixture was boiled in a microwave oven for 5 minutes to dissolve agarose completely. This solution was allowed to cool to 50^0C. 1 µl of ethidium bromide of concentration 10 mg/ml was added to the agarose solution and mixed well. This solution was poured into the tray carefully without forming bubbles. The solution was solidified to form gel and then the comb was removed.

After filling the electrode tank with 1X TBE buffer, the gel casting tray was placed in the electrode tank and the comb was gently removed. 10 µl of 100 bp DNA ladder (Pan Ladder IV) was loaded in the first well and 10 µl of PCR amplified product and 2 µl of 6X gel loading buffer (0.25% bromophenol blue, 0.25% Xyline cyanol and 40% sucrose) were mixed thoroughly and loaded in the wells of agarose gel with a micropipette.

After closing the tank the electrode wires were attached to the power supply. Electrophoresis was done at 78V in 1X TBE buffer. After electrophoresis the gel was taken out and visualized using gel documentation unit (Biorad) and analysed with Quantity 1 programme. Presence or absence of bands was recorded in the form of '0' or '1. The frequency of different bands, their polymorphism and other characters were noted. The polymorphic patterns of the bands produced by each primer were assessed. Gene diversity, genetic similarity, polymorphic loci, percentage polymorphism number of alleles per locus, effective number of alleles per locus and pair wise comparison of similarity index were estimated by POPGENE, version 1.31 software. An Unweighted Pair Group Method with arithmetic mean (UPGMA) dendrogram was constructed using the genetic distance values

Table 1 Sequence and optimum annealing temperature of five primers used for RAPD analysis

Name of primer	Nucleotide length	Sequence	Molecular weight (Da)	Annealing temperature (^{0}C)
OPA1	10mer	CAGGCCCTTC	2963.95	40
OPA3	10mer	AGTCAGCCAC	2997.01	40
OPA7	10mer	GAAACGGGTG	3117.04	39
OPA9	10mer	GGGTAACGCC	3053.00	40
OPA10	10mer	GTGATCGCAG	3068.00	38

3.1.5 Microsatellite

3.1.5.1 DNA samples

DNA samples were extracted by salt precipitation method from pleopods of prawns collected from rivers of Kerala, Orissa and Andhra Pradesh was used for microsatellite analysis.

3.1.5.2 Microsatellite loci

The studies on polymorphism of microsatellite loci Mbr 4 and Mbr 10 were done. The sequence of forward and reverse primers used for both primers and their annealing temperatures are given in Table 2.

3.1.5.3 Preparation of PCR mixture and conditions

DNA fragments were subjected to PCR reaction in a reaction volume of 30 µl. The reagents were 1X PCR buffer, 2mM each of dNTPs, 10 þm of primer, 1.5U Taq polymerase and 50 ng of template DNA

Amplification was done in a gradient thermal cycler (MJ Mini Bio-rad) with a pre denaturation of 3 min. at 94^{0}C followed by 35 cycles of denaturation at 94^{0}C for 30 seconds, appropriate annealing temperature for each primer for 30 seconds and an extension step at 72^{0}C for 1 min., followed by a final

extension of 72^{0}C for 5 min. After amplification the products were stored in -20^{0}C till use.

The quality of DNA was tested on agarose gel before electrophoresis. For this 5 µl of DNA was mixed with gel loading buffer and loaded in the wells of 0.7% agarose gel. Electrophoresis was done under 90 V for 30 min. After electrophoresis the gel was observed the presence of bands in UV transilluminator.

3.1.5.4 Polyacrylamide gel electrophoresis

The glass plates of electrophoresis unit were wiped with absolute ethanol and left to air dry. Spacers were placed in between glass plates. After clipping, the plates were sealed on the sides and bottom with a tape. Sixty ml of 4.5% acrylamide was mixed with 60 µl of TEMED and 60 µl of 0.25 % ammonium persulphate solution. Placed the glass plates at 45^0 angles and carefully filled the gel in between the plates with a syringe without trapping air bubbles. The plates were lowered to allow the gel to fill in between the glass plates and inserted a comb into the gel. The glass plates were kept tightly and allowed the gel to polymerise. After polymerisation the comb was removed from the gel and the PCR products were loaded in the well.

PCR products were subjected to analysis using semi automated genotyping of Hex-labelled primers with a laser driven gel scan system and associated software D-scan.

Table 2 Sequence and optimum annealing temperature of two primers used for microsatellite analysis

Name of primer		Sequence	Annealing temperature (^{0}C)
Mbr4	FP	CCACCTACCGTACATTCCCAAAC	56^{0}C
	RP	CGGGGCGACTTTTAGTATCGAC	
Mbr10	FP	ATGACGATGATGAGGAATGAAGC	56^{0}C
	RP	TTTCAGGCTATATCAAGCAACAG	

3.1.6 Statistical Analysis

Mean values of number of bands, size of band, frequency of band, allelic frequency of band and non bands for each primer were calculated. Allelic frequency of the non bands was calculated as the square root of the frequency of non bands and allelic frequency of the bands was calculated by subtracting the allelic frequency of non band from 1.

Allelic frequency of non band $qi = \sqrt{Qi}$

Where Qi is the frequency of i^{th} non band and qi is the allelic frequency of i^{th} non band.

Allelic frequency of band $pi = 1 - qi$

Where qi is the allelic frequency of non band and pi is the allelic frequency of i^{th} band.

3.2 COMPARATIVE LARVAL PERFORMANCE

Healthy *Macrobrachium rosenbergii* brooders were collected from Periyar of Kerala (KR), Mahanadi of Orissa (OR) and domesticated strains from Andhra Pradesh (AP) were brought to Freshwater prawn hatchery, College of Fisheries, Panangad, Kochi.

3.2.1 Brood Stock Maintenance

Brood stocks were disinfected in the hatchery by placing them in freshwater containing 20 ppm formalin for 30 minutes. After giving formalin treatment, weight and length of brooders were recorded (Table 3). Healthy, active and large sized dark coloured brooders without any damages were placed in oval FRP tanks of 1 ton capacity filled with filtered freshwater. Hideouts were provided in each tank for reducing cannibalism. Mild aeration was also provided with the help of air diffusing stones. After molting, the females were kept along with males in 1:1 ratio. Berried females were kept in

separate tanks filled to a height of 50 cm with filtered and UV treated sea water of salinity 6 ppt, temperature 28-30^{0}C and pH 7.5. Brood stocks were fed at a rate of 1-3% of total biomass with Scampi grower feed (Higashi Premium Scampi feed; Godrej Gold Coin, Mumbai) supplemented with fresh clam meat twice a day in the early morning and late afternoon. Waste was removed from the bottom of the tanks by siphoning and water exchange was done partially. Close monitoring of water quality parameters such as temperature, pH, salinity and dissolved oxygen were checked and recorded daily. Egg diameter (along the long axis) of each berry was measured with an ocular and stage micrometer

Egg diameter = Ocular meter Reading ×Correction Factor.

Table 3 Weight, length and incubation time of brooders of base populations

Andhra Pradesh	Weight (g)		Length (cm)		Incubation time (days)
	Male	Female	Male	Female	
1.	84	74	21.3	20	20
2.	55	42	20.5	15.5	20
3.	43	33	18.5	13.5	19
4.	75	80	19.5	19	20
5.	75	48	20.5	17	19
6.	59	56	22.5	18	19
7.	65	61	19.5	19	19
8.	53	52	21.5	17	20
Orissa	**Weight (g)**		**Length (cm)**		**Incubation time (days)**
1.	118	110.9	21	23.5	19
2.	99.5	92g	20	23	20
3.	85	88	22	22	20
4.	82	90	19	22.6	19
5.	90	85	19	20	19
6.	95	100	20.5	22	19
7.	80	73	20.5	17.5	20
8.	82	85	19.8	18	19
Kerala	**Weight (g)**		**Length (cm)**		**Incubation time (days)**
1.	99.5	104.3	21.6	23	20
2.	100.3	105.25	22	24	19
3.	118.7	111.87	25	24	18
4.	102	95	22	20.4	20
5.	97	87	20	19.8	19
6.	92	84	20.6	19.5	20
7.	95.5	86.4	21.4	21	19
8.	92	76	20.2	18.4	19

Plate. 1 Base population of Andhra Pradesh (AP).

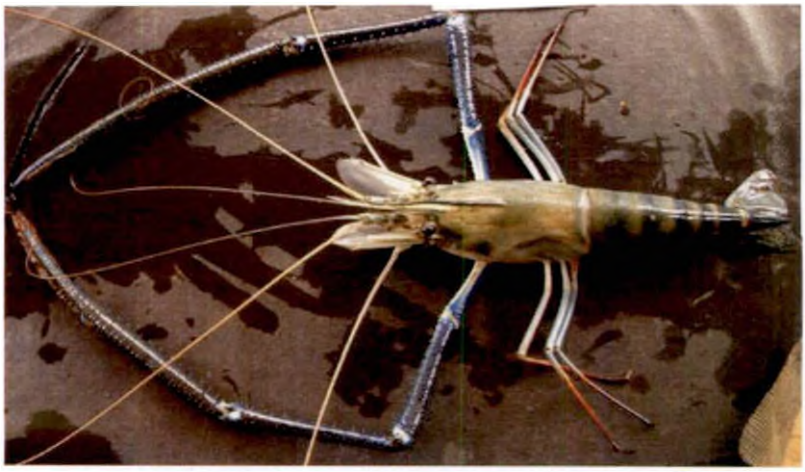

Plate 2. Base population of Kerala (KR)

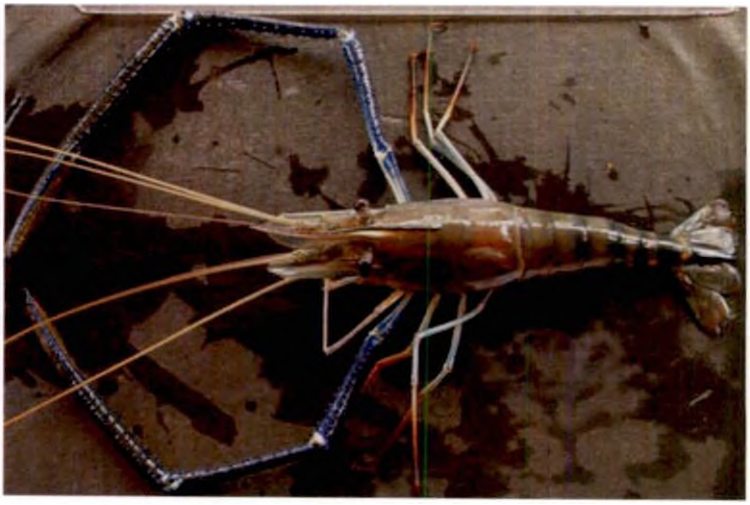

Plate 3. Base population of Orissa (OR).

3.2.2 Hatching

Once the eggs became grey in colour, the tanks were checked daily to confirm hatching. After hatching the brood chambers of the spent females were examined to record partial or complete hatching. The spent females were weighed in an electronic balance of sensitivity 0.02 g. Larvae obtained from the prawns were counted volumetrically and the larval hatch fecundity of different stocks of prawns was estimated (Brooks, 2001).

Hatch fecundity = Number of larvae hatched ÷Weight of spent female in g.

Incubation period of different brooders was also recorded.

3.2.3 Larval Rearing

After hatching, spent prawns were removed from the tank and the counted larvae were kept in 1 ton tank filled with filtered and UV treated sea water of salinity 12 ppt at a density of 50 larvae / litre. Larval count and weight of spent females of each family were recorded. Aeration was also provided in the larval tanks by three air stones equally placed in the tank bottom to maintain high dissolved oxygen and to keep the feed particles suspended in the water. A biological filter of capacity five hundred litre was also connected to each pair of larval rearing tanks. Feeding started from second day onwards. From second to fifth day, newly hatched *Artemia* nauplii was given as feed after separating the shell, five times daily with last and main feed in the evening at the rate of 6 nauplii per ml of water. From fifth day onwards, particulate shrimp and egg custard (200 μ size) were given during day time four times daily and *Artemia* nauplii in the evening. Every day morning and evening after removing the aeration, the sides of the tanks were scrubbed and wastes were siphoned out from the bottom of the tanks. Larvae were collected randomly from each tank and examined under microscope for checking the larval stage and larval rearing period of different stocks by noting the time of appearance of first postlarvae and the time of settlement of all

larvae as postlarvae. The lengths of larvae were randomly checked by using ocular micrometer. 200 numbers of larvae were taken randomly from eight families of three stocks and kept in cylindro-conical tanks under identical condition as that of larval rearing tanks. After larval rearing period survival rate was calculated

Percentage survival = (Number of postlarvae retrieved ÷ Number of larvae stocked) × 100

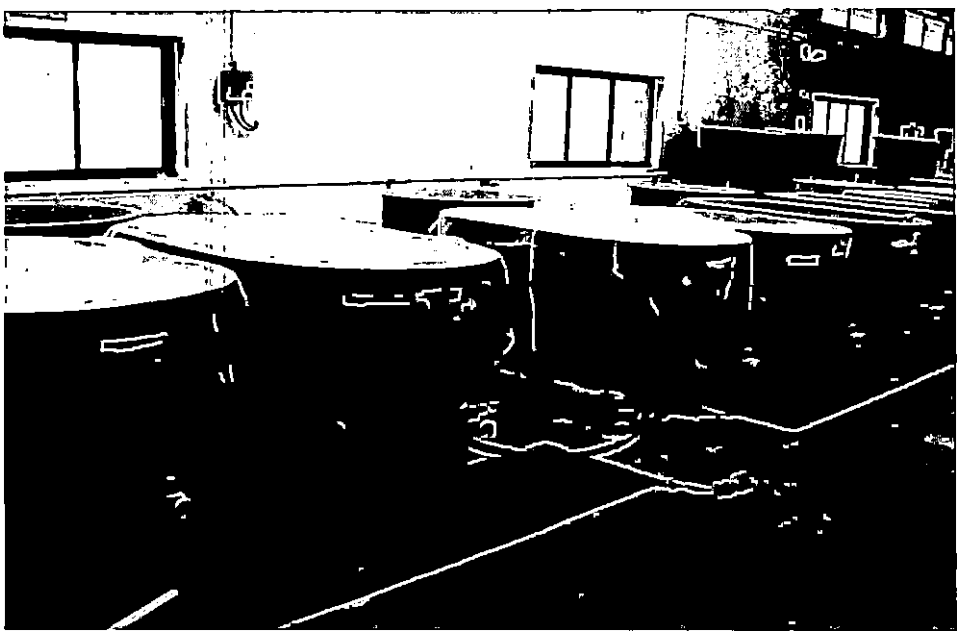

Plate 4 Freshwater prawn hatchery

3.2.4 Stress Tests

M. rosenbergii larvae of Kerala, Orissa and Andhra Pradesh (fifth and tenth stages) were subjected to various stress tests such as salinity, formalin, ammonia and temperature for assessing and comparing its tolerance to various factors.

3.2.4.1 Temperature tolerance test by Incipient Lethal Technique

To determine upper lethal temperature and temperature tolerance of V and X stage of larvae of three different stocks of prawns viz Kerala, Orissa and Andhra Pradesh were abruptly exposed to four different temperatures 35^0C,

37^0C, 39^0C, 41^0C, for a period of one h. by using submersible water heater. A control at 29^0C and six replications for each treatment were maintained for the experiment. Prior to each trial, food was withheld for twenty four hours. Twelve larvae each from three different stocks were kept in one litre beakers filled with brackish water of salinity 12 g l^{-1} and exposed to different temperatures, mild aeration was also provided. Time of death of each larva was recorded. Number of mortalities was taken for calculating the estimated temperatures causing 50% mortality of larvae as the incipient upper lethal temperatures. Statistical analysis was done by using SPFF software. Probit analysis was done for comparing the temperature tolerance of larvae belonging to different stocks at 1% level of significance.

3.2.4.2. Salinity stress test

Eight numbers each of larvae (fifth and tenth stages) were placed in 1 litre beakers and filled with 800 ml of water of different salinities of 12 g l^{-1}, 15 g l^{-1}, 20 g l^{-1},25 g l^{-1}, 30 g l^{-1}, 35 g l^{-1} and 50 g l^{-1} for one hour. Mild aeration was also provided in the beaker. All other parameters such as temperature, pH and DO were kept constant. Six replications were also set for each treatment. Time of mortality of each animal was recorded. Probit analysis was done for determining the lethal concentration and tolerance limit of salinity of different stocks for one h.

3.2.4.3. Formaldehyde stress test

Eight numbers each of larvae (fifth and tenth stages) were placed in 1 litre beaker and filled with 800 ml of water of different concentrations of formaldehyde 0 ppm, 50 ppm, 100 ppm, 150 ppm, 250 ppm and 350 ppm for 3 h. Mild aeration was also provided in the beaker. All other parameters such as temperature, pH and DO were kept constant. Six replications were also set for each treatment. Time of mortality of each animal was recorded. Probit analysis was done for determining the lethal concentration and tolerance limit of formaldehyde of different stocks for three hours.

3.2.4.4. Ammonia stress test

Eight numbers each of larvae (fifth and tenth stages) were placed in one liter beaker and filled with 800 ml of water of different concentrations of ammonia 100, 110, 120, 130, 140, 150, 160 ppm for 6 h. Mild aeration was also provided in the beaker. All other parameters such as temperature, pH (8.3) and DO were kept constant. Six replications were also set for each treatment. Time of mortality of each animal was recorded. Probit analysis was done for determining the lethal concentration and tolerance limit of ammonia of different stocks for six h.

3.2.5. Postlarval Rearing

When majority of larvae become postlarvae, the salinity of water was gradually reduced from 12 g l^{-1} at the rate of 4 g l^{-1} daily to 0 ppt. and percentage survival of larvae were calculated as follows.

Percentage survival= (Number of postlarvae retrieved ÷ Initial Number of post larvae) × 100

M.rosenbergii postlarvae (PL) were reared in large tanks at the rate of 5 nos./l and were fed with prawn starter feed (Higashi Premium Scampi feed; Godrej Gold Coin, Mumbai) along with crushed clam meat four times daily under standard hatchery practices (Sebastian *et al.*, 1993). Twigs and PVC pipes were provided in the tank to increase the surface area, which act as shelter to PL and increase survival rate. Aeration was also provided with air diffusion stones. Water quality parameters were kept optimum during rearing period of two months.

3.3. CAGE CULTURE

Postlarvae of each stock were kept in different tanks for one month and were transferred to separate cages, which were fixed in the pond in such a way as to keep the bottom of the cage in contact with the pond bottom by using

bamboo poles. Counted and weighed postlarvae were stocked in the cages of size 3 × 3 × 1.5m at stocking density of 10 postlarvae/cage. Postlarvae were fed with prawn starter feed (Higashi Premium Scampi feed; Godrej Gold Coin, Mumbai) at the rate of 15% of body weight. Sampling was done fortnightly. During sampling prawns were randomly selected from each cage for assessing its growth and health status. After one month it was transferred to culture ponds of uniform size after assessing growth rate and survival rate.

Plate 5 Cages for nursery rearing

3.4 ASSESSMENT OF ECONOMICALLY IMPORTANT TRAITS OF JUVENILE PRAWNS

3.4.1 Disease Resistance

For checking the disease resistance among three stocks of *M.rosenbergii,* post larvae and juveniles from Kerala, Andhra Pradesh and Orissa stocks were selected. More or less uniform sized postlarvae (0.050 ± .010 g) were challenged with extract of prawns affected with white tail virus and juveniles were subjected to challenge test by using bacteria *Enterococcus aerogens*.

3.4.1.1. Viral challenge study

432 numbers of healthy uniform sized postlarvae having an average weight of 0.050 ± .010 g were selected from six different families of each stock. Eight numbers each were randomly distributed in seventy two glass tanks of size filled with fifty litre of water. Hide outs were provided in the tank to acclimatize the postlarvae for two days. For each stock one tank was kept as control (T_0) and other five were exposed to different concentrations (100 µl, 125 µl, 150 µl, 175 µl and 200 µl) designated by, T_1, T_2, T_3, T_4 and T_5 of viral inoculua with four replications. The experiment was conducted in a randomized block design with six treatments and four replications for each treatment for three stocks. After inoculation mild aeration and feed were also given to the postlarvae. Behavioral changes and appearance of the Postlarvae were observed and the time of mortality was recorded. The dead animals were tested to find out the presence of virus by RT-PCR method.

3.4.1.2 Bacterial challenge study

In this study lyophilized pure culture of *Enterococcus aerogenes* (IMTECH,Chandigarh) was used for inducing disease. Before inoculation it was sub cultured in nutrient broth and incubated at 37^{0}C for 24 hours. After incubation the broth was centrifuged at 7500 rpm for 20 min. at 4^{0}C in a refrigerated centrifuge. After removing the supernatant the precipitate was transferred to 10 ml physiological saline (0.75% NaCl). 1 ml each of solution was transferred to two petri dishes for plate counting and 1 ml for serial dilution. Rest of the solution was used for measuring the absorbance at 601 nm. Serially diluted solutions were also used for plate counting and for measuring the absorbance. 15- 20 ml of melted plate count agar was poured into petri plates and mixed the inocula with the media. After solidification the plates were incubated at 37^{0}C for forty eight h. After incubation the numbers of colonies were counted corresponding to each concentration (Table 4). A trial test was also conducted by injecting bacterial solutions of different concentrations to test animals at the rate of 125 µl and the

effect was observed. From the trial test, inoculum containing bacteria at a concentration of 1.1×10^2 cells/ml was selected for conducting challenge study.

Healthy uniform sized juvenile prawns having average weight of 2.5 ± 0.25g were selected from six different families of each stock for challenge study. For this the stock solution of *E.aerogenes* (1.1×10^6cells/ml) and its serial dilutions 10^{-1} to 10^{-4} (T_1, T_2, T_3, T_4 and T_5) were prepared with physiological saline. Acclimatised animals were injected with aliquot of stock and serially diluted bacterial inocula at the rate of 125 µl. Control (T_0) animals were injected with 125 µl sterile physiological saline. The animals were continuously observed for next forty eight h.

3.4.1.3 Haemocyte count

Glass tanks of size 45×30×30 cm were used for this experiment. Healthy uniform sized juvenile prawns having average weight of 2.5 ± 0.25g were selected from seven different families of each stock. Eight numbers each were randomly distributed in tanks filled with fifty litre of water for acclimatisation. Aeration and hide outs were provided in the tank to acclimatize the juveniles for two days. For each stock, one tank was kept as control and other six were exposed to microorganisms of concentration (1.1×10^2 cells/ml). The experiment was conducted in a randomized block design with two treatments and seven replications for each treatment for three stocks.

After six h prawns were collected from each tank and haemolymph was drawn by using 1 ml syringe. Before collecting the haemolymph the syringe was rinsed with anticoagulant Anderson solution (Table 5) and the haemolymph was mixed with twice the volume of anticoagulant. Haemocytes were counted by checking the number of haemocytes presents in boxes of four corners of haemocytometer (Rohem, India).

3.4.1.4. Prophenoloxidase activity (PPO)

After six h exposure to *E.aerogenus* (1.1×10^2 cells/ml), hemolymph samples were collected from the ventral sinus cavity of prawn (T_1). Samples were left to clot at $-25^0C\pm 2^0C$ for 1 h before being transferred to -80^0C for 5 min. and thawed to induce lysis of cells. The liquid fraction hemocyte lysate fraction (HLF) was collected.

Phenoloxidase activity in hemolymph samples were determined using L- dihydroxy phenyl alanine (L-DOPA) as a substrate (Soderhall, 1983). TBS (30 µl) was added to the experimental cuvette containing 30µl hemolymph sample. Then 60 µl L-DOPA (1.6 mg/ml in TBS) was added followed by immediate mixing. Then 2 ml of TBS was added as diluents and enzyme activity was determined by measuring the absorbance of dopa chrome at 490 nm against a blank as control (C) containing 260 µl TBS and 60 µl L-DOPA. Absorbance values at 1 and 3 min after addition of 200 µl TBS were recorded. The enzyme activity was expressed as unit, defined as the amount of enzyme giving an increase in absorbance at 490 nm.

Table 4 Absorbance value of serially diluted inocula and corresponding number of micro organisms

Dilution	Absorbance	No. of Microorganisms
10^{-1}	0.910	-
10^{-2}	0.84	-
10^{-3}	0.092	1.8×10^7
10^{-4}	0.009	1.0×10^6
10^{-5}	0.003	1.1×10^5

Table 5 Composition of Anderson solution

Ingredients	Concentrations
Sodium citrate	27 mM
Sodium chloride	336 mM
EDTA	9.00 mM
Glucose	115 mM

3.4.2. Carcass composition

Carcass proximate analyses were done for evaluating the nutritional quality of three different stocks of *M. rosenbergii* juveniles. For biochemical analysis, minced meat of *M. rosenbergii* juveniles of weight 3±0.28g were selected from six different families of each stock. Percentage moisture content was estimated by drying the samples at 105^0C till a constant weight was arrived. Crude percentage protein by Microkjeldhal's method (AOAC, 1984), percentage of crude fat by solvent extraction method by using petroleum ether (BP. 40-60^0C) and percentage ash content of the samples by burning the samples at 550^0C±10^0C for 6 h. in a muffle furnace were estimated. Carbohydrate was estimated by difference method (Hasting, 1976).

3.4.3 Pond Culture

M.rosenbergii juveniles of different families of three stocks were stocked at the rate of 600 numbers in separate ponds of uniform size (20×8 m) to avoid mixing and to minimize environmental effect of variation. Each treatment group of animals were fed with scampi grower feed (Higashi Premium Scampi feed; Godrej Gold Coin, Mumbai) twice daily at the rate of 5% of biomass. Sampling was done fortnightly. During each sampling, the weights of prawns were checked to adjust the feed ration. Temperature of pond water was checked daily while dissolved oxygen, ammonia and pH were monitored at weekly intervals. Period of grow out culture was 5 months. At the end of the experiment, prawns were harvested after complete draining of the pond. Each family was separately kept in different labeled tanks. Growth rate, survival rate percentage morphotypes, and head - tail ratio were estimated.

Plate 6 Different stocks of *M. rosenbergii* stocked in uniform sized ponds

3.4.4. Percentage of Morphotype

All the prawns from each stock were sorted to different morphotypes such as blue clawed male (BC), orange clawed male (OC), small males (runts) and females. Percentages of each morphotypes in three stocks were estimated.

3.4.5 Head- Tail Ratio

Random samples of hundred prawns from each family of three stocks were collected and weight of head and tail were measured. Head - tail ratio of each individual was estimated.

3.5 SELECTIVE BREEDING

3.5.1 Selection and Maintenance of Brood Stocks

Based on the superior qualities estimated according to trait analysis data of three stocks, families were selected. Female and male parents were chosen carefully based on the characteristics described by Sagi and Ra'anan (1985) to

maximize mating success. Healthy active and large dark coloured male *M. rosenbergii* having thick dark blue claws without any damages and females with orange coloured ovaries were collected from selected families for cross breeding between three stocks such as Andhra Pradesh, Kerala and Orissa. Single male and females were kept isolated, at a density of 10 nos. / m^3 in separate tanks. The tanks were filled with sand-filtered and aerated tap water. Hide outs were provided in each tank for reducing cannibalism. Mild aeration was also provided with the help of air diffusing stones and a temperature of 27 $\pm 0\ 2^0$C were maintained throughout the study. The animals were fed daily with commercial pelleted feed (Higashi Premium Scampi feed; Godrej Gold Coin, Mumbai) during day time and fresh clam meat during night at a rate of 5% of the animal's body weight, according to recommendations of Moreno *et al.* (2000). Excess feed and waste were removed by daily water exchange of half the total volumes.

3.5.2 Mating Design and Production of Full Sib Groups

Newly moulted mature females were placed into the FRP tanks with mature males in 1:1 ratio and were monitored for mating and spawning. Breeding pairs formed between three different stocks are given below:

Andhra Pradesh (male) × Orissa (female)	APOR,
Orissa (male) × Andhra Pradesh (female)	ORAP,
Andhra Pradesh (male) × Kerala (female)	APKR,
Kerala (male) × Andhra Pradesh (female)	KRAP,
Orissa (male) × Kerala (female)	ORKR,
Kerala (male) × Orissa (female)	KROR.

Eight replications for each breeding pairs were kept for breeding. Berried females were kept in separate tanks filled to a height of 50 cm with filtered and UV treated sea water of salinity 6 ppt, temperature 28-30^{0}C and pH 7.5. Brood stocks were fed at a daily rate of 1-3% of total biomass with Scampi grower feed (Higashi Premium Scampi feed, Godrej Gold Coin, Mumbai) at the rate of 1-3% of total feed and fresh clam meat twice a day in the early morning and late afternoon. Waste was removed from the bottom of the tank by siphoning and water exchange was done partially. Close monitoring of water quality parameters such as temperature, pH, salinity and dissolved oxygen were done and recorded daily. Egg diameter and hatch fecundity of each brooders were estimated as earlier in section.

Larval rearing, cage culture and grow out culture of F1 generations of different stocks were done as in the case of base population.

3.5.3 Evaluation of Genetic Improvement

The phenotypic performance of F1 generation of each combination were compared after estimating larval rearing period, survival rate, stress tolerance and growth rate. But cage culture, grow out culture and assessment of other important traits such as disease resistance, growth rate, percentage morphotype and head- tail ratio were done only for hybrids APKR, APOR, and ORKR, which showed better survival rate. The procedure for the assessment of economically important traits of F1 hybrids was as in the case of parent population. Heritability of weight of three hybrids was estimated by simple linear regression method. Average weight of eight parents and mean weight of 100 offspring from corresponding families were taken for heritability calculation.

3.6 STATISTICAL ANALYSIS

Statistical analysis was done by using Genstat -Seventh Edition (DE3), version: 7.2.2.222, VSN international Ltd. By using this software one way and

two way analysis were done for data. In viral and bacterial challenge study square root method was used to transform data before analysis. Simple linear regression method was used to analyse the heritability of weight of three hybrids.

3.7 WATER QUALITY

During the experiment water quality parameters such as Temperature, pH, Salinity Dissolved Oxygen and NH_3-N were checked periodically in hatchery and in cages Temperature, pH, Salinity and Dissolved Oxygen were checked by the following methods done and recorded daily (Table 6,7).

Temperature	:	By using Mercury thermometer of 0.10^0C
pH	:	By using universal indicator solution.
Dissolved Oxygen	:	By using DO meter (SD Tech, Mumbai)
NH_3-N	:	By using Ammonia tetra kit

Table 6 Water quality parameters of larval rearing of *M. rosenbergii.*

Water quality parameters	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Temperature (^{0}C)	28±0.05	27±0.09	28±0.03	28.0.07	27±0.06	28±0.03	28±0.02	28.±0.06
pH	8.3±0.2	8.3±0.3	8.3±0.2	8.3±0.4	8.3±0.3	8.3±0.2	8.3±0.4	8.3±.0.4
DO (mgL^{-1})	8.55±0.05	8.45±0.06	8.30±0.05	8.10±0.02	8.11±0.07	8.23±0.04	8.00±0.11	8.00±0.15
NH_3-N (mgL^{-1})	0.044±0.007	0.045±0.003	0.030±0.003	0.040±0.004	0.033±0.001	0.035±0.001	0.038±0.020	0.036±0.007
Salinity (gl^{-1})	12	12	12	12	12	12	12	12

Table 7 Water quality parameters of postlarval rearing of *M. rosenbergii*.

Water quality parameters	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Temperature (^{0}C)	28±0.04	28±0.06	28±0.03	28.0.06	28±0.06	28±0.05	28±0.04	28.±0.05
pH	8.3±0.2	8.3±0.2	8.3±0.2	8.3±0.3	8.3±0.3	8.3±0.4	8.3±0.4	8.3±.0.3
DO (mgL^{-1})	8.55±0.05	8.55±0.05	8.35±0.05	8.60±0.22	8.30±0.07	8.35±0.04	8.30±0.06	8.20±0.16
NH_3-N (mgL^{-1})	0.040±0.006	0.042±0.003	0.035±0.003	0.040±0.002	0.035±0.003	0.035±0.002	0.035±0.022	0.035±0.004

Results

4. RESULTS

4.1 GENETIC CHARACTERIZATION.

4.1.1 RAPD Markers

Random amplified polymorphic DNA was used to study the genetic diversity among three populations of *Macrobrachium rosenbergii* from Andhra Pradesh, Orissa and Kerala. Eighty two numbers of samples were genetically characterized using five RAPD markers.

4.1.2 Yield and Quality of DNA

The yield and purity of DNA extracted from different samples from different regions are given in Tables 8 and 9. Quality of DNA was checked by the presence of a single high molecular weight band on agarose gel.

The average of DNA obtained from Andhra, Orissa and Kerala samples were 1219.69, 1199.531 and 1110.310ng /µl respectively. The ratio of optical density at 260 nm and 280 nm was taken as a measure of quality of DNA. The average value of optical density for Andhra Pradesh, Orissa and Kerala prawns were 1.821±0.063, 1.8 ±0.046 and 1.81 ± 0.043 respectively.

Table 8 Concentrations and purity of DNA of Kerala (KR) stocks of *M.rosenbergii*

Sl. No	Stock	Conc.ng/µl	260/280	Sl. No	Stock	Conc.ng/µl	260/280
1.	KR1	2253	1.87	16.	KR16	1300	1.80
2.	KR2	2253	1.80	17.	KR17	1145	1.80
3.	KR3	2234	1.80	18.	KR18	1123	1.78
4.	KR4	1437	1.87	19.	KR19	1870	1.87
5.	KR5	668	1.88	20.	KR20	344	1.77
6.	KR6	1170	1.96	21	KR21	987	1.80
7.	KR7	1497	1.89	22.	KR22	1123	1.78
8.	KR8	1273	1.82	23.	KR23	129	1.77
9	KR9	313	1.84	24.	KR24	960	1.82
10.	KR10	1122	1.82	25.	KR25	1126	1.80
11.	KR11	1132	1.83	26.	KR26	1276	1.83
12.	KR12	1145	1.80	27.	KR27	1111	1.82
13.	KR13	1245	1.82	28	KR28	1100	1.83
14.	KR14	1234	1.76	29.	KR29	1233	1.80
15.	KR15	1267	1.85	30	KR30	1023	1.77
31.	KR31	1122	1.85	32	KR30	1170	1.87

KR1-KR11-Achankovil river; KR12-KR19- Chalakudi river
KR20-KR27- Murinjapuzha; KR28-KR32- Valapattanam

Table 9 Concentration and Purity of DNA of Andhra Pradesh (AP) and Orissa (OR) stocks of *M.rosenbergii*

Sl. No	Stock	Conc. ng /µl	260/280	Stock	Conc.ng /µl	260/280
1.	A1	160	1.88	O1	307	1.77
2.	A2	1320	1.88	O2	450	1.96
3.	A3	2080	1.81	O3	393	1.85
4.	A4	3240	1.75	O4	710	1.7
5.	A5	1800	1.64	O5	440	1.82
6.	A6	830	1.86	O6	810	1.81
7.	A7	1670	1.89	O7	917	1.8
8.	A8	959	1.9	O8	605	1.82
9	A9	638	1.65	O9	1204	1.74
10.	A10	1810	1.84	O10	2200	1.73
11.	A11	1250	1.80	O11	1289	1.80
12.	A12	960	1.82	O12	1278	1.79
13.	A13	930	1.80	O13	1189	1.82
14.	A14	555	1.83	O14	1111	1.78
15.	A15	1111	1.88	O15	956	1.82
16.	A16	1350	1.83	O16	1290	1.80
17.	A17	1290	1.90	O17	1230	1.78
18.	A18	1200	1.80	O18	1235	1.84
19.	A19	1003	1.79	O19	2340	1.80
20.	A20	1810	1.84	O20	2200	1.73
21.	A21	890	1.79	O21	1490	1.78
22.	A22	888	1.78	O22	1237	1.80
23.	A23	590	1.83	O23	987	1.79
24.	A24	1200	1.80	O24	950	1.83
25.	A25	1400	1.91	O25	945	1.82
26.	A26	1200	1.84	O26	1185	1.82
27.	A27	1304	1.84	O27	1183	1.80
28.	A28	988	1.82	O28	1020	1.80
29.	A29	945	1.82	O29	1048	1.80

A1- A29- Andhra Pradesh; O1-O29- Orissa

4.1.3 Optimization of PCR Parameters

RAPD-PCR conditions were optimized to get maximum amplification and to minimize the production of incomplete non specific amplified products. The PCR was done in 25 µl volume as per the concentrations and conditions given below:

Parameters/Variables	Level
1. Template DNA	20 ng/µl
2. Primer	10 pmols
3. dNTP mix	0.5mM
4. Taq polymerase	3U
5. 1X Taq buffer (10mM Tris, 15 mM $MgCl_2$,Gelatin)	
6. Deionised water	

Cycle parameters

Initial denaturation	95 ^{0}C for 3 min.
Denaturation	94 ^{0}C for 1 min.
Annealing	suitable temperature for 1 min
Extension	72 ^{0}C for 1.3 min
Final extension	72 ^{0}C for 10 min.
	Total 39 cycles

4.1.4 Primers Used

Twelve random oligonucleotide primers were used for the amplification of three DNA samples. Based on the intensity, clarity and polymorphism of bands obtained five primers were selected for RAPD analysis.

4.1.5 Number of Bands

The band size obtained in RAPD analysis ranged from 150 to 2550 bp and the average numbers of bands produced by the primers are given in Table 10. Scoring was done, based on bands within molecular weight of 500-1000 bp generated by each primer. (Plate 7-11).

Table 10 RAPD primers and average number of bands amplified by the primers

Primers	Andhra Pradesh	Orissa	Kerala
OPA1	6.64 ± 1.08	5.12 ± 0.93	8.38 ± 1.24
OPA3	6.76± 0.78	5.04 ± 0.54	4.94 ± 1.08
OPA7	6.08±0.86	6.56 ± 0.87	4.88±1.52
OPA9	1.20 ± 0.65	2.32 ± 0.63	4.22 ± 1.31
OPA10	9.04 ± 0.79	6.88 ± 0.44	6.56 ± 0.88

4.1.6 Frequency of Bands

Frequency of each band was determined from the ratio of the number of animals having the bands (n) to the total number of animals (N) screened. The frequency of different bands was found out for each primer.

4.1.7 Allelic Frequency of Bands

Allelic frequency of bands was calculated from the band frequency. Due to the dominant nature of RAPD marker both homozygous and heterozygous alleles are shown as single band. Allelic frequencies were calculated based on the assumption that there are only two alleles at the same locus.

4.1.8 Primer wise Results

The DNA samples of three stocks of prawns were analysed with five primers and classification of bands based on their frequency are presented in the Table 11.

Table 11 Classification of bands identified by amplification with different RAPD primers based on their frequency in Andhra population, Orissa and Kerala.

Primers	Population	No. of bands and allele frequency				Total bands
		<0.25	0.25-0.50	0.50-1	1	
OPA1	AP	5	1	3	3	12
	OR	6	0	4	1	11
	KR	4	1	4	2	11
OPA3	AP	2	0	6	1	9
	OR	4	0	3	2	9
	KR	1	5	4	0	10
OPA7	AP	3	0	6	0	9
	OR	2	0	6	1	9
	KR	5	2	4	0	11
OPA9	AP	3	0	0	1	4
	OR	5	0	0	2	7
	KR	1	4	3	0	8
OPA10	AP	4	0	5	5	14
	OR	2	0	3	5	10
	KR	4	3	2	4	13

4.1.8.1 Primer OPA1

Amplification of DNA of three populations of prawns is given in Plate 7, which revealed the presence of twelve amplified products in Andhra population and eleven bands each for OR and KR populations. Bands OPA1i, OPA1j and OPA1l are present in all the samples of Andhra Pradesh. OPA1c is present in all Orissa prawns, while OPA1c and OPA1d were present in all

Kerala samples. The rare bands with frequencies less than 0.25 were five, six and four for Andhra Pradesh, Orissa and Kerala respectively. Name of bands, approximate size of the products, frequency of the band, frequency of the band allele and frequency of non band alleles of Andhra Pradesh, Orissa and Kerala were given in Tables 12, 13 and 14 respectively.

Table 12 Name, approximate size, frequency of bands and the alleles produced by the primer OPA1 on DNA samples of prawns of Andhra Pradesh.

Sl. No.	Name of band	Approximate size in base pairs	Frequency of bands	Frequency of band alleles	Frequency of non band alleles
1	OPA1a	200	0.920	0.717	0.283
2	OPA1b	250	0.920	0.717	0.283
3	OPA1c	275	0.040	0.020	0.980
4	OPA1d	300	0.960	0.800	0.200
5	OPA1e	325	0.000	0.000	1.000
6	OPA1f	350	0.040	0.020	0.980
7	OPA1g	375	0.000	0.000	1.000
8	OPA1h	400	0.120	0.062	0.938
9	OPA1i	550	1.000	1.000	0.000
10	OPA1j	600	1.000	1.000	0.000
11	OPA1k	650	0.280	0.151	0.849
12	OPA1l	700	1.000	1.000	0.000
13	OPA1m	750	0.120	0.062	0.938
14	OPA1n	800	0.080	0.041	0.959
15	OPA1o	850	0.160	0.083	0.917

Table 13 Name, approximate size, frequency of bands and the alleles produced by the primer OPA1 on DNA samples of prawns of Orissa.

Sl. No.	Name of band	Approximate size in base pairs	Frequency of bands	Frequency of band alleles	Frequency of non band alleles
1	OPA1a	200	0.040	0.020	0.980
2	OPA1b	250	0.080	0.041	0.959
3	OPA1c	275	1.000	1.000	0.000
4	OPA1d	300	0.960	0.800	0.200
5	OPA1e	325	0.000	0.000	1.000
6	OPA1f	350	0.000	0.000	1.000
7	OPA1g	375	0.000	0.000	1.000
8	OPA1h	400	0.920	0.717	0.283
9	OPA1i	550	0.880	0.654	0.346
10	OPA1j	600	0.120	0.062	0.938
11	OPA1k	650	0.000	0.000	1.000
12	OPA1l	700	0.840	0.600	0.400
13	OPA1m	750	0.120	0.062	0.938
14	OPA1n	800	0.040	0.020	0.980
15	OPA1o	850	0.120	0.062	0.938

Table 14 Name, approximate size, frequency of bands and the alleles produced by the primer OPA1 on DNA samples of prawns of Kerala

Sl. No.	Name of band	Approximate size in base pairs	Frequency of bands	Frequency of band alleles	Frequency of non band alleles
1	OPA1a	200	0.313	0.171	0.829
2	OPA1b	250	0.094	0.048	0.952
3	OPA1c	275	0.063	0.032	0.968
4	OPA1d	300	0.938	0.750	0.250
5	OPA1e	325	0.750	0.500	0.500
6	OPA1f	350	0.719	0.470	0.530
7	OPA1g	375	0.656	0.414	0.586
8	OPA1h	400	1.000	1.000	0.000
9	OPA1i	550	0.063	0.032	0.968
10	OPA1j	600	0.094	0.048	0.952
11	OPA1k	650	1.000	1.000	0.000
12	OPA1l	700	1.000	1.000	0.000
13	OPA1m	750	0.906	0.694	0.306
14	OPA1n	800	0.000	0.000	1.000
15	OPA1o	850	1.000	1.000	0.000

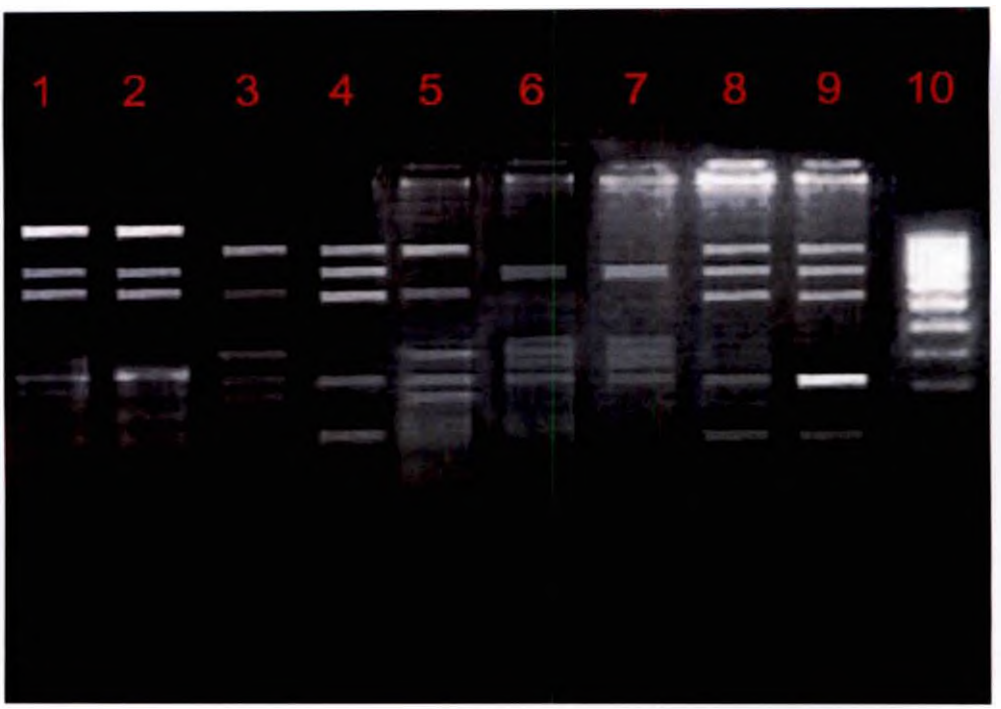

Plate. 7 .RAPD bands obtained from *M. rosenbergii* using OPA1 primer.

1-AP; 2-AP; 3-OR; 4-KAK; 5-OR;6-KCH; 7-KCH; 8-KAK; 9-KAK; 10-M.

4.1.8.2 Primer OPA3

Amplification of DNA of three populations of prawns is given in Plate 8, which revealed the presence of nine amplified products in Andhra Pradesh and Orissa. But in Kerala ten bands were present with OPA3 primer. Band OPA3b, was present in all the samples of Andhra Pradesh. OPA3f and OPA3i were present in all Orissa prawns, while no common bands were present in all Kerala samples. The rare bands with frequencies less than 0.25 were two, four and one for Andhra Pradesh, Orissa and Kerala respectively. Name of bands, approximate size of the products, frequency of the band, frequency of the band allele and frequency of non band alleles of Andhra Pradesh, Orissa and Kerala were given in Tables 15, 16 and 17 respectively.

Table 15 Name, approximate size, frequency of bands and the alleles produced by the primer OPA3 on DNA samples of prawns of Andhra Pradesh.

Sl. No.	Name of band	Approximate size in base pairs	Frequency of bands	Frequency of band alleles	Frequency of non band alleles
1	OPA3a	100	0.960	0.800	0.200
2	OPA3b	200	1.000	1.000	0.000
3	OPA3c	250	0.080	0.041	0.959
4	OPA3d	350	0.080	0.041	0.959
5	OPA3e	400	0.960	0.717	0.283
6	OPA3f	500	0.920	0.800	0.200
7	OPA3g	600	0.960	0.960	0.040
8	OPA3h	700	0.920	0.717	0.283
9	OPA3i	800	0.000	0.000	1.000
10	OPA3j	1000	0.880	0.654	0.346

Table 16 Name, approximate size, frequency of bands and the alleles produced by the primer OPA3 on DNA samples of prawns of Orissa.

Sl. No.	Name of band	Approximate size in base pairs	Frequency of bands	Frequency of band alleles	Frequency of non band alleles
1	OPA3a	100	0.000	0.000	1.000
2	OPA3b	200	0.080	0.041	0.959
3	OPA3c	250	0.040	0.020	0.980
4	OPA3d	350	0.040	0.020	0.980
5	OPA3e	400	0.920	0.717	0.283
6	OPA3f	500	1.000	1.000	0.000
7	OPA3g	600	0.040	0.020	0.980
8	OPA3h	700	0.960	0.800	0.200
9	OPA3i	800	1.000	1.000	0.000
10	OPA3j	1000	0.960	0.800	0.200

Table 17 Name, approximate size, frequency of bands and the alleles produced by the primer OPA3 on DNA samples of prawns of Kerala.

Sl. No.	Name of band	Approximate size in base pairs	Frequency of bands	Frequency of band alleles	Frequency of non band alleles
1	OPA3a	100	0.281	0.152	0.848
2	OPA3b	200	0.156	0.081	0.919
3	OPA3c	250	0.250	0.134	0.866
4	OPA3d	350	0.313	0.171	0.829
5	OPA3e	400	0.406	0.229	0.771
6	OPA3f	500	0.875	0.646	0.354
7	OPA3g	600	0.906	0.694	0.306
8	OPA3h	700	0.750	0.500	0.500
9	OPA3i	800	0.594	0.363	0.637
10	OPA3j	1000	0.406	0.229	0.771

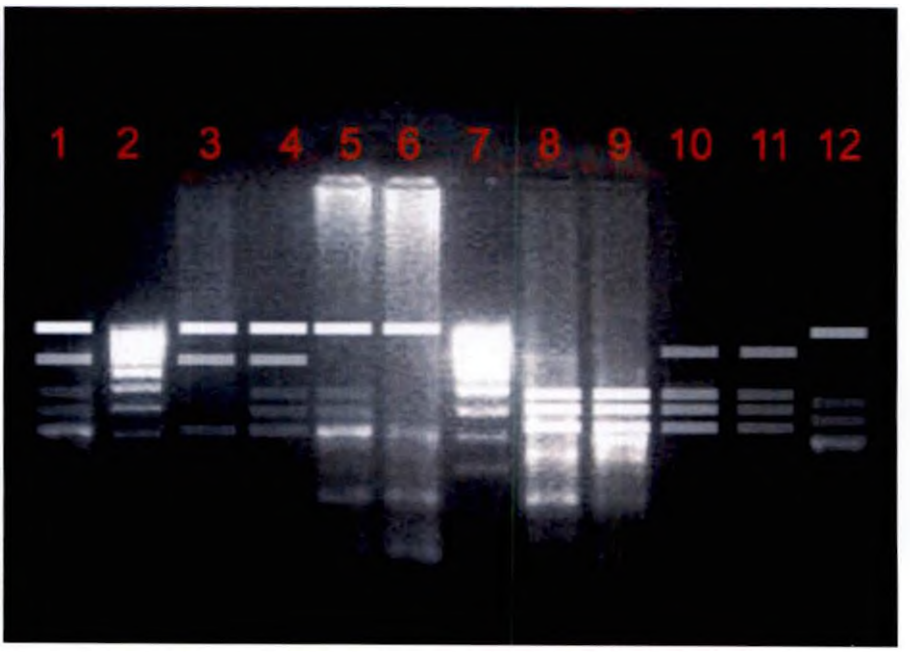

Plate 8 RAPD bands obtained from *M. rosenbergii* using OPA3 primer.

1-KCH; 2-M; 3-OR; 4-KCH; 5-AP; 6-AP; 7-M; 8-KKA; 9-KKA; 10-KM; 11-KM; 12-AP.

4.1.8.3 Primer OPA7

Amplification of DNA of three populations of prawns is given in Plate 9, which revealed the presence of nine amplified products for Andhra and Orissa and eleven bands for Kerala population. No common band was present in all the samples of Andhra Pradesh. The condition was same in samples from Kerala also. OPA7k was present in all Orissa prawns. The rare bands with frequencies less than 0.25 were three numbers for Andhra Pradesh, two for Orissa and five for Kerala respectively. Name of bands, approximate size of the products, frequency of the band, frequency of the band allele and frequency of non band alleles of Andhra Pradesh, Orissa and Kerala were given in Tables 18, 19 and 20 respectively.

Table 18 Name, approximate size, frequency of bands and the alleles produced by the primer OPA7 on DNA samples of prawns of Andhra Pradesh.

Sl. No.	Name of band	Approximate size in base pairs	Frequency of bands	Frequency of band alleles	Frequency of non band alleles
1	OPA7a	250	0.080	0.041	0.9596
2	OPA7b	275	0.000	0.000	1.000
3	OPA7c	300	0.120	0.062	0.938
4	OPA7d	350	0.960	0.800	0.200
5	OPA7e	400	0.920	0.717	0.283
6	OPA7f	450	0.960	0.800	0.200
7	OPA7g	500	0.000	0.000	1.000
8	OPA7h	600	0.960	0.800	0.200
9	OPA7i	700	0.960	0.800	0.200
10	OPA7j	800	0.960	0.800	0.200
11	OPA7k	1000	0.040	0.020	0.980

Table 19 Name, approximate size, frequency of bands and the alleles produced by the primer OPA7 on DNA samples of prawns of Orissa.

Sl. No.	Name of band	Approximate size in base pairs	Frequency of bands	Frequency of band alleles	Frequency of non band alleles
1	OPA7a	250	0.920	0.717	0.283
2	OPA7b	275	0.080	0.0408	0.959
3	OPA7c	300	0.920	0.717	0.283
4	OPA7d	350	0.040	0.020	0.980
5	OPA7e	400	0.920	0.717	0.283
6	OPA7f	450	0.000	0.000	1.000
7	OPA7g	500	0.880	0.654	0.346
8	OPA7h	600	0.880	0.880	0.120
9	OPA7i	700	0.880	0.880	0.120
10	OPA7j	800	0.000	0.000	1.000
11	OPA7k	1000	1.000	1.000	0.000

Table 20 Name, approximate size, frequency of bands and the alleles produced by the primer OPA7 on DNA samples of prawns of Kerala.

Sl. No.	Name of band	Approximate size in base pairs	Frequency of bands	Frequency of band alleles	Frequency of non band alleles
1	OPA7a	250	0.094	0.905	0.095
2	OPA7b	275	0.344	0.190	0.810
3	OPA7c	300	0.938	0.750	0.250
4	OPA7d	350	0.156	0.081	0.919
5	OPA7e	400	0.125	0.065	0.935
6	OPA7f	450	0.094	0.048	0.952
7	OPA7g	500	0.875	0.646	0.354
8	OPA7h	600	0.094	0.048	0.952
9	OPA7i	700	0.906	0.693	0.307
10	OPA7j	800	0.344	0.048	0.952
11	OPA7k	1000	0.906	0.694	0.3062

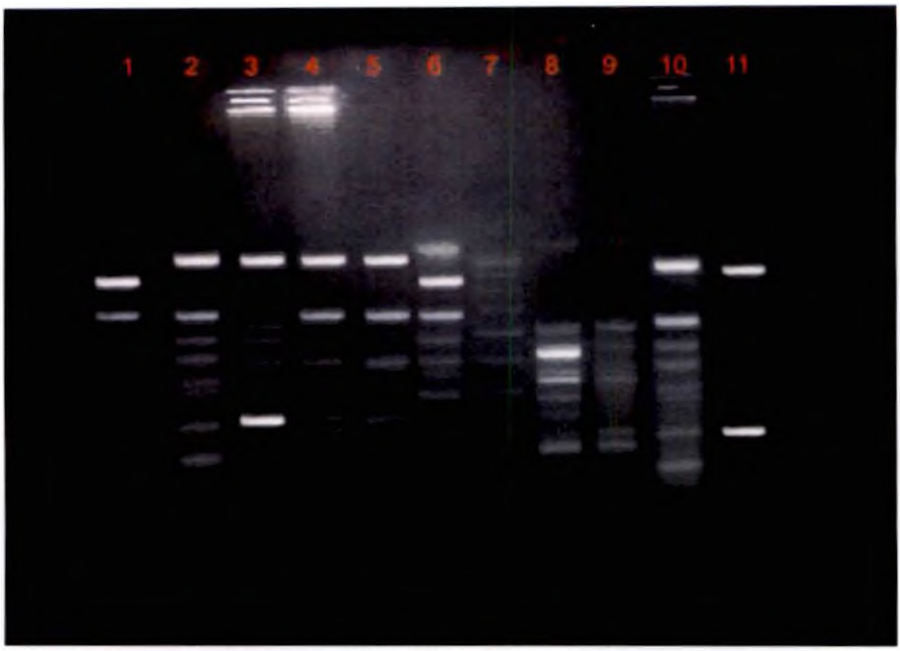

Plate 9 RAPD bands obtained from *M. rosenbergii* using OPA7 primer.

1-AP; 2-OR; 3-KAK; 4-KCH; 5-KKA; 6-AP; 7-M; 8-AP; 9-OR; 10-OR; 11-AK

4.1.8.4 Primer OPA9

Amplification of DNA of three populations of prawns is given in Plate 10, which revealed the presence four amplified products for Andhra Pradesh, seven for Orissa and nine for Kerala with the primer OPA9. Three of the bands of Andhra and five of Orissa showed frequencies less than 0.25. While OPA9h of Andhra sample and OPA9i and OPA9j bands of Orissa sample showed a band frequency of one. In Kerala sample this primer exhibited band frequency less than 0.25 was one in number. Name of bands, approximate size of the products, frequency of the band, frequency of the band allele and frequency of non band alleles of Andhra Pradesh, Orissa and Kerala were given in Tables 21, 22 and 23 respectively.

Table 21 Name, approximate size, frequency of bands and the alleles produced by the primer OPA9 on DNA samples of prawns of Andhra Pradesh.

Sl. No.	Name of band	Approximate size in base pairs	Frequency of bands	Frequency of band alleles	Frequency of non band alleles
1	OPA9a	200	0.080	0.041	0.959
2	OPA9b	275	0.040	0.020	0.980
3	OPA9c	300	0.000	0.000	1.000
4	OPA9d	350	0.000	0.000	1.000
5	OPA9e	400	0.080	0.041	0.959
6	OPA9f	475	0.000	0.000	1.000
7	OPA9g	500	0.000	0.000	1.000
8	OPA9h	650	1.000	1.000	0.000
9	OPA9i	750	0.000	0.000	1.000
10	OPA9j	1000	0.000	0.000	1.000

Table 22 Name, approximate size, frequency of bands and the alleles produced by the primer OPA9 on DNA samples of prawns of Orissa.

Sl. No.	Name of band	Approximate size in base pairs	Frequency of bands	Frequency of band alleles	Frequency of non band alleles
1	OPA9a	200	0.080	0.080	0.920
2	OPA9b	275	0.080	0.080	0.920
3	OPA9c	300	0.040	0.040	0.960
4	OPA9d	350	0.000	0.000	1.000
5	OPA9e	400	0.040	0.040	0.960
6	OPA9f	475	0.080	0.080	0.920
7	OPA9g	500	0.000	0.000	1.000
8	OPA9h	650	0.000	0.000	1.000
9	OPA9i	750	1.000	1.000	0.000
10	OPA9j	1000	1.000	1.000	0.000

Table 23 Name, approximate size, frequency of bands and the alleles produced by the primer OPA9 on DNA samples of prawns of Kerala.

Sl. No.	Name of band	Approximate size in base pairs	Frequency of bands	Frequency of band alleles	Frequency of non band alleles
1	OPA9a	200	0.875	0.646	0.354
2	OPA9b	275	0.375	0.209	0.791
3	OPA9c	300	0.125	0.000	0.935
4	OPA9d	350	0.438	0.250	0.75
5	OPA9e	400	0.344	0.190	0.810
6	OPA9f	475	0.281	0.152	0.848
7	OPA9g	500	0.313	0.441	0.559
8	OPA9h	650	0.750	0.500	0.500
9	OPA9i	750	0.000	0.000	1.000
10	OPA9j	1000	0.531	0.315	0.685

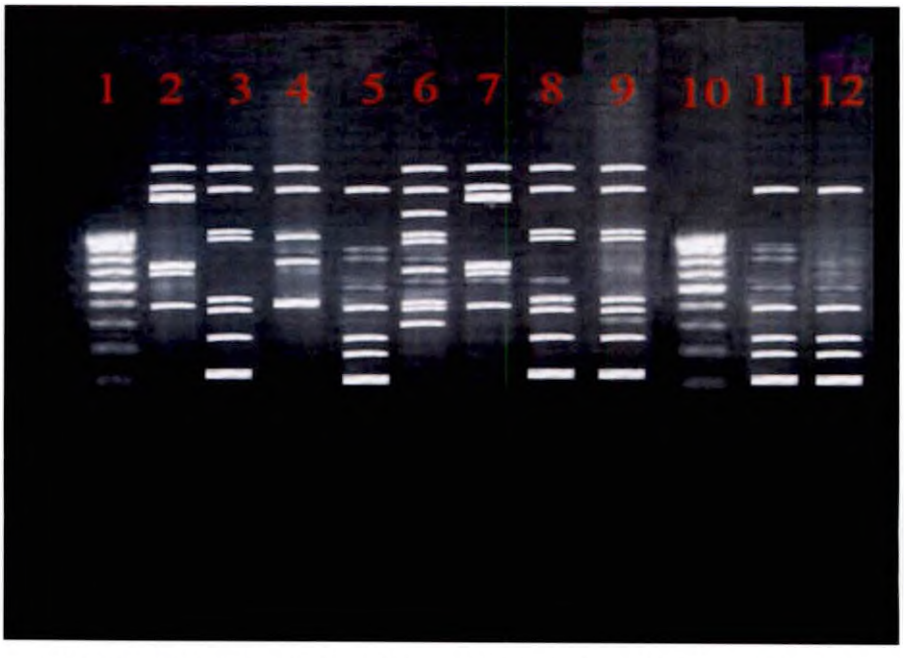

Plate 10 RAPD bands obtained from *M. rosenbergii* using OPA9 primer.

1-M; 2-AP; 3-KCH; 4-OR; 5-KAK; 6-KM; 7-AP; 8-OR; 9-OR; 10-M; 11-KCH; 12-KCH

4.1.8.5 Primer OPA10

Primer OPA10 yielded fourteen bands for Andhra population, ten bands for Orissa population and thirteen for Kerala population. This primer produced four rare bands for Andhra population, two for Orissa and four for Kerala and showed sufficient polymorphism in Andhra and Kerala populations. Name of bands, approximate size of the products, frequency and allelic frequency of bands and allelic frequency of non bands of Andhra Pradesh, Orissa and Kerala samples are given in Tables 24, 25 and 26 respectively.

Table 24 Name, approximate size, frequency of bands and the alleles produced by the primer OPA10 on DNA samples of prawns of Andhra Pradesh

Sl. No.	Name of band	Approximate size in base pairs	Frequency of bands	Frequency of band alleles	Frequency of non band alleles
1	OPA10a	100	0.960	0.800	0.200
2	OPA10b	150	1.000	1.000	0.000
3	OPA10c	175	0.960	0.800	0.200
4	OPA10d	225	0.000	0.000	1.000
5	OPA10e	250	0.000	0.000	1.000
6	OPA10f	275	0.000	0.000	1.000
7	OPA10g	300	1.000	1.000	0.000
8	OPA10h	350	1.000	1.000	0.000
9	OPA10i	375	1.000	1.000	0.000
10	OPA10j	400	0.960	0.800	0.200
11	OPA10k	500	0.040	0.020	0.980
12	OPA10l	550	0.080	0.041	0.959
13	OPA10m	600	1.000	1.000	0.000
14	OPA10n	650	0.200	0.106	0.894
15	OPA10o	750	0.840	0.600	0.400
16	OPA10p	800	0.160	0.083	0.917
17	OPA10q	850	0.880	0.654	0.346

Table 25 Name, approximate size, frequency of bands and the alleles produced by the primer OPA10 on DNA samples of prawns of Orissa.

Sl. No.	Name of band	Approximate size in base pairs	Frequency of bands	Frequency of band alleles	Frequency of non band alleles
1	OPA10a	100	0.960	0.800	0.200
2	OPA10b	150	1.000	1.000	0.000
3	OPA10c	175	0.000	0.000	1.000
4	OPA10d	225	1.000	1.000	0.000
5	OPA10e	250	1.000	1.000	0.000
6	OPA10f	275	0.040	0.020	0.980
7	OPA10g	300	0.000	0.000	1.000
8	OPA10h	350	0.000	0.000	1.000
9	OPA10i	375	0.040	0.020	0.980
10	OPA10j	400	0.960	0.800	0.2000
11	OPA10k	500	0.000	0.000	1.000
12	OPA10l	550	0.000	0.000	1.000
13	OPA10m	600	1.000	1.000	0.000
14	OPA10n	650	0.000	0.000	1.000
15	OPA10o	750	0.880	0.654	0.346
16	OPA10p	800	0.000	0.000	1.000
17	OPA10q	850	1.000	1.000	0.000

Table 26 Name, approximate size, frequency of bands and the alleles produced by the primer OPA10 on DNA samples of prawns of Kerala

Sl. No.	Name of band	Approximate size in base pairs	Frequency of bands	Frequency of band alleles	Frequency of non band alleles
1	OPA10a	100	1.000	1.000	0.000
2	OPA10b	150	1.000	1.000	0.000
3	OPA10c	175	0.063	0.388	0.612
4	OPA10d	225	0.000	0.000	1.000
5	OPA10e	250	0.960	0.800	0.200
6	OPA10f	275	0.250	0.134	0.866
7	OPA10g	300	0.962	0.805	0.195
8	OPA10h	350	0.125	0.065	0.935
9	OPA10i	375	0.094	0.048	0.952
10	OPA10j	400	1.000	1.000	0.000
11	OPA10k	500	0.000	0.000	1.000
12	OPA10l	550	0.000	0.000	1.000
13	OPA10m	600	0.313	0.171	0.829
14	OPA10n	650	0.080	0.041	0.959
15	OPA10o	750	0.480	0.279	0.721
16	OPA10p	800	0.000	0.000	1.000
17	OPA10q	850	1.000	1.000	0.000

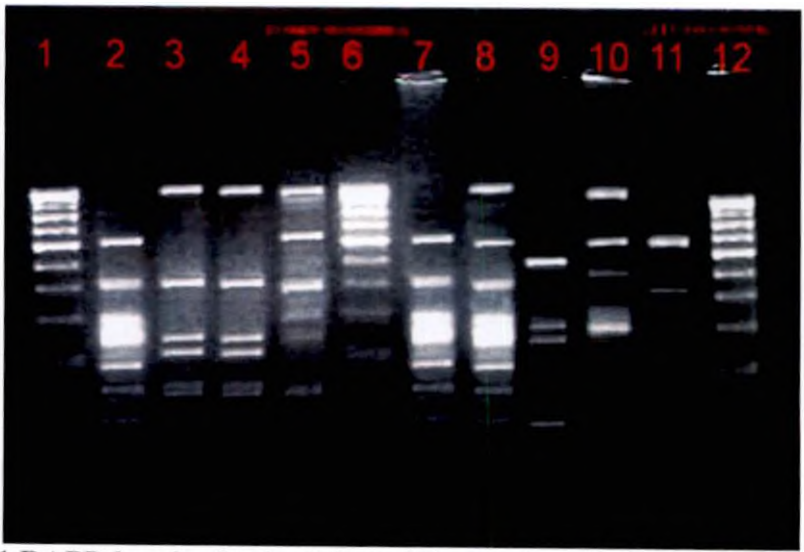

Plate 11 RAPD bands obtained from M. rosenbergii using OPA10 primer.

1-M; 2-AP; 3-OR; 4-OR; 5-KCH; 6-M; 7-AP; 8- AP; 9-KAK; 10-KCH; 11-KKA; 12-M.

4.1.9 Analysis of Six Populations

Samples of KR population were collected from four different sites. In the first analysis Kerala samples were considered as four populations (KAK, KCH, KVA and KM) and overall six populations were analysed. The overall estimate of gene diversity (h), genetic similarity, Shannon Information Index, number of polymorphic loci and percentage of polymorphism in all the six populations of *M.rosenbergii* were estimated and are given in Table 27. Among KR samples KRAK showed maximum percentage polymorphism (55.17 %) and KM showed lowest. In this study the highest genetic diversity (h) was found within KAK (0.17) samples, followed by KCH samples (0.16). Lowest heritability was found in KM samples.

For all the above populations, the average expected heterozygosity or Nei's gene diversity value 'h' (Nei, 1978) was 0.33± 0.20. The overall percentage of polymorphic loci was 89.66 and total number of polymorphic loci was 26. The observed number of alleles was 1.90 and effective number of alleles was 1.61. Gene diversity value (Gst) and gene flow among populations were 0.57 and 0.38 respectively.

Shannon diversity index was calculated to get relative estimate of the degree of variation within each population. The Shannon Index ranged from 0.09 (KM) to 0.26 (KAK). Maximum I value was found in KAK (0.26) and the minimum for KM population (0.09). Maximum number of polymorphic loci was found in AP (17) and the minimum in KM (5). Maximum percentage polymorphism was observed in AP (58.62 %) followed by KAK (55.17 %) and the least was found in KM (17.24%).

Table 27 Estimate of gene diversity, genetic similarity, average pair wise similarity index, number of polymorphic loci and percentage polymorphism in six populations of *M.rosenbergii*

Parameter	AP	OR	KAK	KCH	KVA	KM
Gene diversity(h)	0.16±0.17	0.13± 0.19	0.17±0.19	0.16±0.21	0.12 ±0.19	0.06 ±0.15
Shannon Information index (I)	0.25±0.25	0.20±0.27	0.26±0.28	0.24±0.30	0.17±0.28	0.09±0.22
No. of polymorphic loci	17	13	16	13	9	5
Percentage polymorphic	58.62 %	44.83 %	55.17 %	44.83 %	31.03 %	17.24 %

Average pair wise similarity index (SI) and genetic distance based on Nei's unbiased measures of genetic identity and genetic distance was calculated for five primers together and are given in Table 28. The values of Nei's unbiased genetic distance (D) ranged from 0.06 to 0.55 among populations. The highest genetic distance was observed between the samples collected from AP and KM, while the genetic distance between the samples collected from KAK and KVA was found to be the minimum. An Unweighted Pair Group Method with arithmetic mean (UPGMA) dendrogram was constructed using the genetic distance values to show the genetic relationships among the six populations of *M. rosenberii* using POPGEN Software (Figure 1).

Table 28 Pair wise comparison of similarity index (above diagonal) and genetic distance below diagonal of *M.rosenbergii* (in six populations) based on Nei (1978), calculated for 5 primers

Sites	AP	OR	KAK	KCH	KVA	KM
AP	****	0.6732	0.7582	0.6633	0.7083	0.5753
OR	0.3956	****	0.6683	0.7834	0.6769	0.6537
KRAK	0.2768	0.4031	****	0.8765	0.9392	0.8548
KCH	0.4105	0.2442	0.1318	****	0.8867	0.8369
KVA	0.3448	0.3903	0.0627	0.1202	****	0.8332
KM	0.5529	0.4251	0.1569	0.1780	0.1825	****

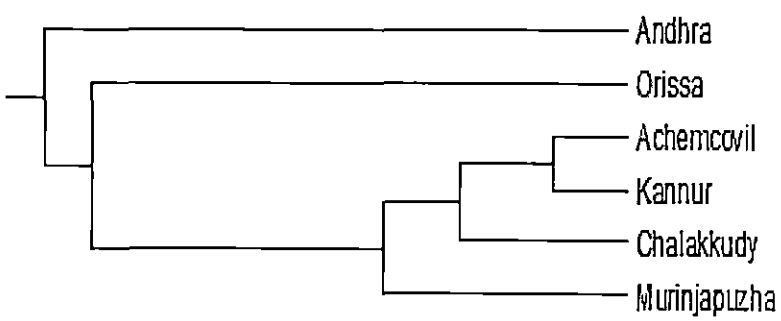

Figure1 Dendrogram constructed using the genetic distance values of six populations of *M.rosenbergii*.

4.1.10 Analysis of Three Populations

Gene diversity (h), genetic similarity, Shannon Information Index, number of polymorphic loci and percentage of polymorphism in all the three populations of *M.rosenbergii* were estimated and are given in Table 29. Among three samples highest gene diversity was shown by KR (0.21) followed by AP (0.16) and least diversity among OR (0.13).

For the above three populations, the average expected heterozygosity or Nei's gene diversity value 'h' was 0.33±0.20. The overall Shannons diversity (I) was 0.48±0.27. The overall percentage of polymorphic loci was 89.66 and total number of polymorphic loci was 26. The observed number of alleles was 1.90 ± 0.31 and effective number of alleles was 1.61± 0.41.

Shannons diversity was calculated to get relative estimate of the degree of variation within each population. Maximum value of Shannon Information Index (I) was observed in the population KR followed by AP and least in OR. Highest number of polymorphic loci was observed for KR (18) followed by AP and OR. Highest percentage polymorphism was found in KR population (62.07%) and lower in OR (44.83 %).

Table 29 Estimate of gene diversity, Shannon information index, no. of polymorphic loci and percentage polymorphism in three populations of *M.rosenbergii*

Parameter	AP	OR	KR
Gene diversity(h)	0.16±0.17	0.13± 0.19	0.21 ±0.21
Shannon Information Index (I)	0.25±0.25	0.20±0.27	0.31±0.29
No. of polymorphic loci	17	13	18
Percentage polymorphism	58.62 %	44.83 %	62.07%

Average pair wise similarity index (SI) and genetic distance based on Nei's unbiased measures of genetic identity and genetic distance was calculated for five primers together and are given in Table.30. The values of Nei's unbiased genetic distance D ranged from among populations with 0.32 to 0.39. The highest genetic distance was observed between the samples collected from AP and OR. The genetic distance between samples collected from AP as well as KR was found to be minimum. The genetic distance between AP and KR was found to be same as that of the genetic distance between AP and OR. An Unweighted Pair Group Method with arithmetic mean (UPGMA) dendrogram was constructed using the genetic distance values to show the genetic relationships among the six populations of *M.rosenberii* using popgene Software (Figure.2).

Table 30 Pair wise comparison of similarity index (above diagonal) and genetic distance below diagonal of three populations *M.rosenbergii* (three populations) based on Nei (1978), for 5 primers

Sites	AP	OR	KR
AP	****	0.68	0.72
OR	0.39	****	0.73
KR	0.32	0.32	****

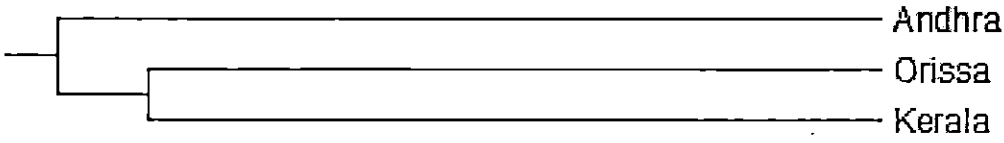

Figure2 Dendrogram constructed using the genetic distance values of three populations of *M.rosenbergii*.

4.1.11 Microsatellite

Preliminary studies of genetic polymorphism of *M.rosenbergii* from Kerala, Orissa and Andhra Pradesh were done with microsatellite marker. A total of 82 DNA samples were isolated from pleopods of three populations of prawns.

Microsatellite profile of *M.rosenbergii* at Mbr 4 and Mbr 10 are given in plates 12 and 13. Further studies are needed with more primers for the analysis of results.

4.1.12 PCR Primers

Two microsatellite loci (Mbr 4 and Mbr 10) were selected for this study. Forward primers of the two primers were labelled with Rex.

4.1.13 PCR Conditions

The DNA samples were subjected to PCR reaction in a volume of 30µl. The reaction mixture contain 1X PCR buffer, 2mM each of dNTPs, 10 þm of primer, 1.5 U Taq polymerase and 50 ng of template DNA.

PCR conditions were standardised and amplification was done with pre denaturation of 3 min. at 94^0C followed by 35 cycles of denaturation at 94^0C for 30 seconds, annealing at 56^0Cfor each primer for 30 seconds and an extension step at 72^0C for 1 min., followed by a final extension of 72^0C for 5 minutes.

4.1.14 Size and Number of Alleles

Number of alleles produced and size range of alleles were found for by Andhra, Orissa and Kerala populations (Table 31). Mbr 4 produced 20 (216-250 bp), 19 (206-240 bp) and 21 (210-300 bp) alleles for Andhra, Orissa and Kerala populations respectively. Mbr10 produced 26, 29 and 51 numbers of alleles of size range 150-252 bp, 150-250 bp and 150-252 bp for Andhra, Orissa and Kerala populations respectively.

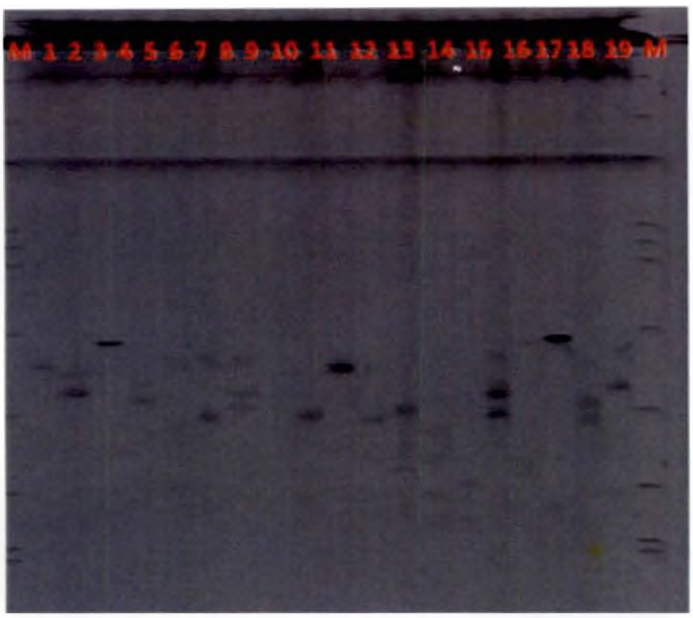

Plate 12 Microsatellite profile of *M.rosenbergii* at loci Mbr 4 collected from KR, AP and OR.

M -Marker; 1- Or4; 2- Or3; 3- Or2;4- Or1;5-Ap5;6- Ap4;7-Ap3;8- Ap2; 9- Ap1; 10-Ak3;11-Ak2; 12- Ak1; 13-Ch7; 14- Ch5; 15- Ch3; 16- Ch1; 17-Va9; 18- Va8; 19- Va5; M

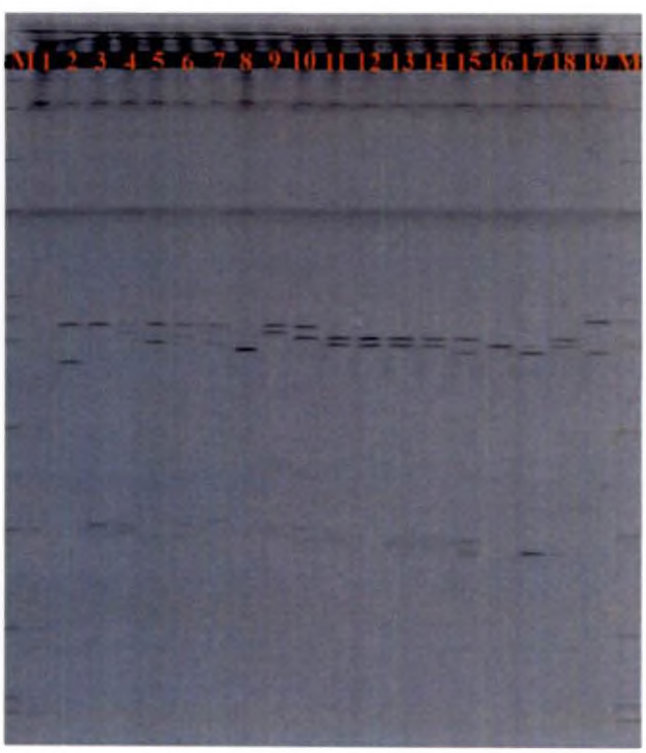

Plate 13 Microsatellite profile of *M.rosenbergii* at loci Mbr10 collected from KR, AP and OR

M -Marker; 1- Or4; 2- Or3; 3- Or2;4- Or1;5-Ap5;6- Ap4;7-Ap3;8- Ap2; 9- Ap1; 10-Ak3;11-Ak2; 12- Ak1; 13-Ch7; 14- Ch5; 15- Ch3; 16- Ch1; 17-Va9; 18- Va8; 19- Va5; M

Table 31 Number of observations, number of alleles and size range, of Andhra, Orissa and Kerala prawns.

Sl.No	Populations	Parameters	Mbr4	Mbr10
1	No. of observations	Andhra	13	11
2		Orissa	15	16
3		Kerala	18	20
4	No. of alleles	Andhra	20	26
5		Orissa	19	29
6		Kerala	21	61
7	Size range of alleles (bp)	Andhra	216-250	150-252
8		Orissa	206-240	150-260
9		Kerala	210-300	150-252

4.2 INDICES OF LARVAL PERFORMANCE

4.2.1 Egg Diameter

In this experiment the egg diameters of three populations from Andhra Pradesh (AP), Orissa (OR) and Kerala (KR) and their six hybrids (APOR, APKR, KROR, ORAP, KRAP and ORKR) were estimated. The results are given in Table 32 and graphically presented in Figure 3. From the Analysis of Variance (Table 33) it was found that the egg diameter of *M. rosenbergii* was significantly different ($p < 0.001$) among populations and hybrids. Highest egg diameter (757.23mm) was observed in the hybrid KROR this was on par with OR, KR and ORKR, but significantly different from other populations and hybrids. The hybrid KRAP showed the lowest diameter (732.65mm) and was on par with ORAP and APOR.

4.2.2 Larval Hatch Fecundity

Hatch fecundity of three base populations and their hybrids were estimated and are summarised in Table 32. Graphical presentation of the same is given in Figure 4. These results showed that highest hatch fecundity was for Kerala stock (1081 no./g) and lowest for KRAP (850 no./g). Analysis of Variance (Table 34) and LSD test revealed that the hatch fecundity of Kerala (KR) was on par with the other parent populations AP, OR, and the hybrid KROR. KRAP and ORAP are two hybrids showing minimum hatch fecundities, which are significantly lower than other populations and hybrids ($p<0.005$).

4.2.3 Larval Rearing Period

Mean larval rearing period of three base populations and their hybrids are given in Table 32. From results it is clear that the least larval rearing period was for APKR (23 days) followed by AP (28 days) and the highest for OR (43 days). Graphical presentation of larval rearing periods of different populations and hybrids is shown in Figure 5. Analysis of Variance (Table35) of data on larval rearing period of the prawns showed significant difference ($p<0.001$) among the different populations and hybrids. LSD test (Table 14) revealed significant difference ($p<0.05$) among all pairs except (KR, KRAP) and (KRAP, KROR).

4.2.4 Larval Survival

The data on mean survival rate of three base populations and hybrids are given in Table32. Highest survival rate was recorded for APOR (80.62%) followed by AP (80.38 %). Lowest survival rate was for KRAP (22.75 %). Graphs of larval survival rate of different populations and hybrids are shown in Figure 6. Analysis of Variance (Table 18) and LSD test (Table 36) were carried out and the results showed that APOR was on par with AP. Similarly OR was on par with KR and KROR.

Table 32 Egg diameter, hatch fecundity, larval rearing period and larval survival rate of different populations and hybrids

Population	Egg diameter (mm)	Hatch fecundity (no./g)	Larval rearing period (days)	Larval survival rate (%)
Andhra(AP)	748.07 ^{b} ± 3.06	1046.75 ^{a} ±87.85	27.63 ^{b} ±3.29	80.38 ^{f} ±1.60
Orissa (OR)	756.40 ^{c} ±8.63	1050.38 ^{a} ±74.89	42.88 ^{h} ±2.98	73.00 ^{e} ±2.20
Kerala(KR)	753.07 ^{bc} ±7.12	1081.38 ^{a} ±41.28	34.62 ^{d} ±1.69	72.75 ^{e} ±1.28
APOR	737.65 ^{a} ±5.84	992.88 ^{b} ± 37.48	42.12 ^{g} ±1.36	80.62 ^{f} ±1.06
APKR	748.48 ^{b} ±9.43	1012.63 ^{b} ± 25.0	23.12 ^{a} ±1.13	65.00 ^{d} ±1.31
KROR	757.23 ^{c} ±8.06	1041.13 ^{ab} ±59.47	36.62 ^{e} ± 0.92	72.62 ^{e} ±1.69
ORAP	734.65 ^{a} ±2.16	920.75 ^{c} ±24.41	38.25 ^{f} ± 0.46	30.75 ^{b} ±2.19
KRAP	732.65 ^{a} ±1.54	850.13 ^{d} ±29.33	36.38 ^{de} ± 0.74	22.75 ^{a} ±1.17
ORKR	754.32 ^{bc} ±8.31	1020.25 ^{b} ±64.57	32.12 ^{c} ±1.13	32.75 ^{c} ±1.28
LSD (0.05)	**7.338**	**52.41**	**1.817**	**1.425**

Values in the table are mean±SD of 8 replications; Mean values with the same or common superscript do not differ significantly ($p<0.05$).

Table 33. ANOVA of egg diameter of different populations and hybrids

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability (p).
Replication	7	217.62	31.09	0.58	<.001
Population and hybrids	8	5862.18	732.77	13.65*	
Residual	56	3005.41	53.67		
Total	71	9085.20			

*Significant ($p<0.001$)

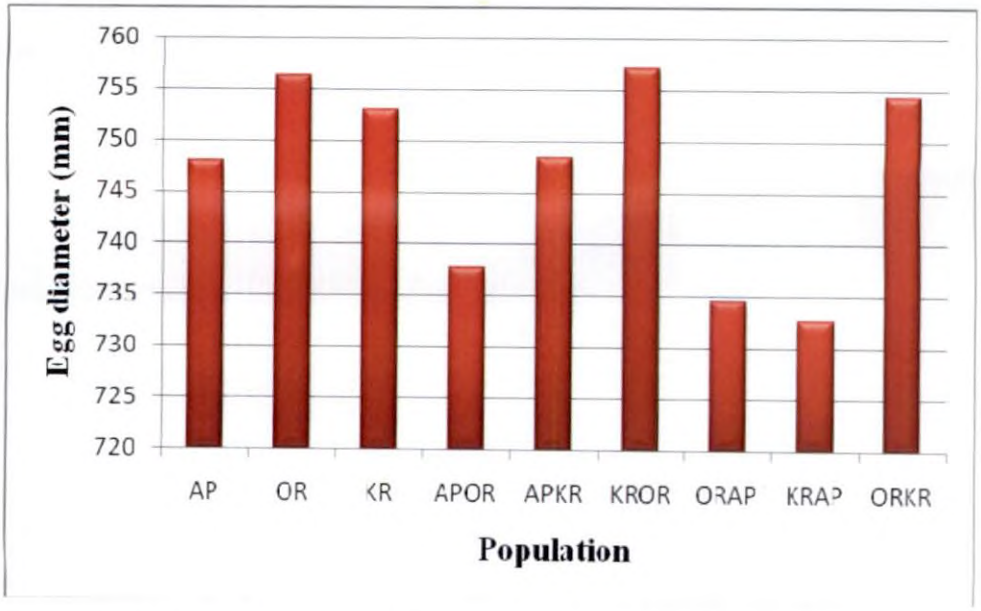

Fig.3. Egg diameter of different populations and hybrids of *M. rosenbergii*

Table 34 ANOVA of larval hatch fecundity of different populations and hybrids

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability (p)
Replication	7	30255	4322	1.58	< .001
Population and hybrids	8	338963	42370	15.48*	
Residual	56	153304	2738		
Total	71	522521			

*Significant ($p<0.001$)

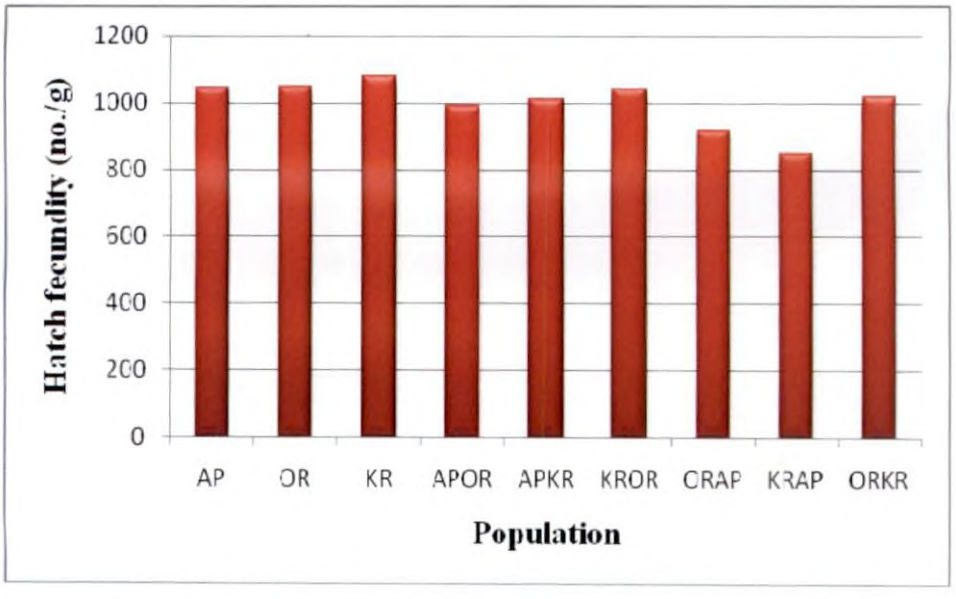

Fig.4. Larval hatch fecundity of different populations and hybrids of *M. rosenbergii*

Table 35. ANOVA of larval rearing period of different populations and hybrids

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability (p)
Replication	7	16.17	2.31	0.70	<.001
Population and hybrids	8	2652.11	331.51	100.71*	
Residual	56	184.33	3.29		
Total	71	2852.61			

*Significant ($p<0.001$)

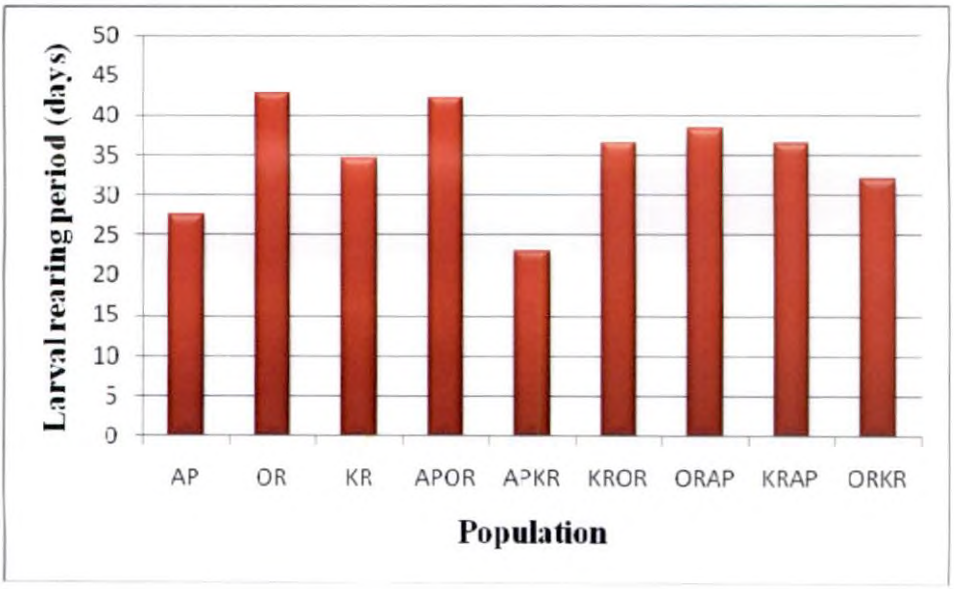

Fig.5. Larval rearing period of different populations and hybrids of *M. rosenbergii*

Table 36 ANOVA of larval survival rate of different populations and hybrids.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	probability. (p)
Replication	7	44.21	6.32	3.12	<.001
Population and hybrids	8	34659.25	4332.41	2139.15*	
Residual	56	113.42	2.03		
Total	71	34816.88			

*Significant ($p<0.001$)

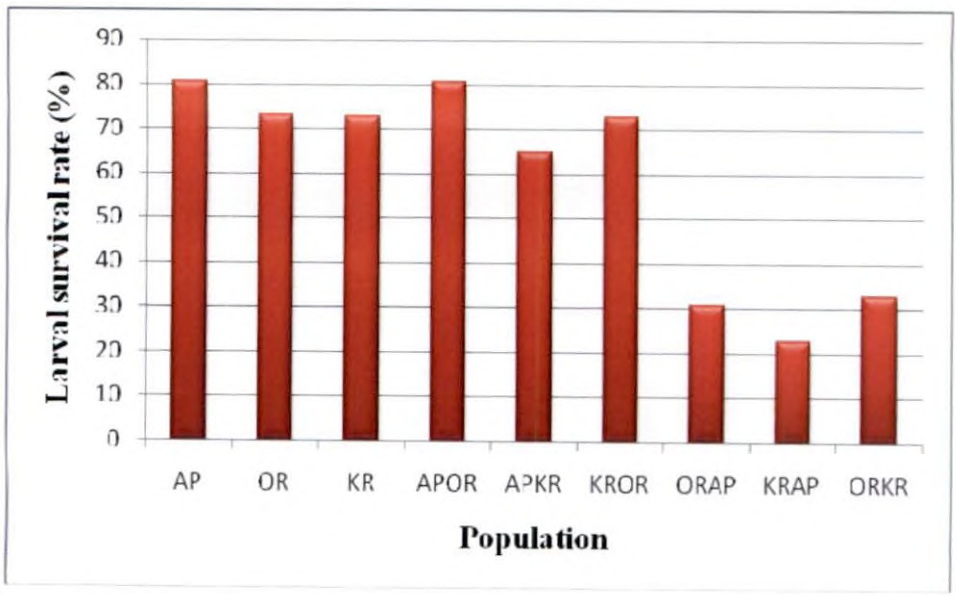

Fig.6. Larval survival rate of different populations and hybrids of *M.rosenbergii*

Table 37 Length of larval stages of *M.rosenbergii* of different populations

Stage / Population	Length of larval stages (mm)												
	1	2	3	4	5	6	7	8	9	10	11	PL	Mean±SD
AP	2519.2	2760.7	3074.4	3289.7	3627.8	5235.0	5724.3	6867.4	8633.0	8901.8	9743.6	10793.7	5930.9^{e} ±2982.44
OR	2644.3	2875.6	3471.5	3750.8	3999.2	4436.3	6295.0	8858.0	9635.3	10170.8	11616.9	12035.7	6649.12^{f}±3576.60
KR	2701.8	2956.8	3078.4	3944.8	4838.5	5239.7	6199.2	8964.1	9449.5	10504.2	11741.9	12175.4	6816.19^{g}±3554.12
APOR	2181.7	2281.7	2485.9	2738.1	3973.7	4571.8	7084.7	8143.3	8533.0	8970.6	9439.4	9966.1	5864.17^{d}±3102.62
APKR	2100.5	2285.9	2421.3	2992.3	3652.8	3946.6	4446.7	5417.8	6286.7	7266.0	8041.2	8518.4	4781.35^{b}±2290.38
KROR	2271.3	2731.8	3136.0	3617.4	5021.9	5699.1	7453.6	8699.7	9756.1	10658.4	11533.6	12312.5	6907.62^{b}±3634.49
ORAP	2210.9	2304.4	2486.0	2796.4	3792.4	4673.8	5886.6	7428.6	8207.9	8589.2	8999.7	9697.8	5589.48^{c}±2884.76
KRAP	2154.6	2279.6	2410.9	2606.8	2913.0	3577.8	4098.8	4928.1	5855.3	7136.9	8085.0	8443.4	4540.85^{a}±2321.16
ORKR	2277.5	2611.0	2992.2	3532.0	4198.8	5001.0	5719.9	6470.1	7553.6	8845.5	9804.1	10710.5	5809.68^{d}±2888.19
Mean ±SD	2340.2^{j} ±222.96	2565.28 ^{j} ±279.67	2839.62^{k} ±392.35	3252.03^{l} ±487.02	4002.0^{m} ±639.46	4709.01^{n} ±668.88	5878.^{o} ±1088.49	7308.57^{p}± 1496.97	8212.27^{q} ±1410.5	9004.8^{r} ±1277.91	9889.4^{s} ±1449.73	10517.06^{t}± 1485.91	

Values in rows are Mean±SD of twelve replications and values in columns are Mean±SD of nine replications.

Mean values with same or common superscript in a row or column do not differ significantly.

4.2.5 Larval Length

The data on mean length of larvae of different populations at twelve stages are given in Table 37. From the table it is clear that the least larval length was for the population KRAP with 4540.8 mm followed by APKR (4781.3 mm). The highest value was observed for the hybrid KROR (6907.6 mm) followed by KR (6816.2 mm). Similarly, as expected, lowest larval length is at first stage of larvae and highest at twelfth stage. Larval length for various populations and stages are diagrammatically shown in Fig. 7 and Fig. 8. Analysis of variance (Table 38) of data on larval length of prawn showed that the mean larval length was significantly different among populations and among stages ($p<0.001$). The highest larval length was noticed for the hybrid KROR and it was significantly different from other populations. ORKR and APOR showed no significant variation, but all other populations were different from each other. But in the case of stages it was found that there was significant variation between stages

Table 38 ANOVA of larval length of *M.rosenbergii* for different population and stages.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability (p)
Replication	7	3.25	0.4639	12.13	
Populations and hybrids	8	539.2	67.4	1761.63*	<.001
Stage	11	7156	650.5	17003.94*	<.001
Populations and hybrids*stage	88	275.8	3.134	81.91*	<.001
Residual	749	28.66	0.03826		
Total	863	8003			

*Significant ($p<0.001$)

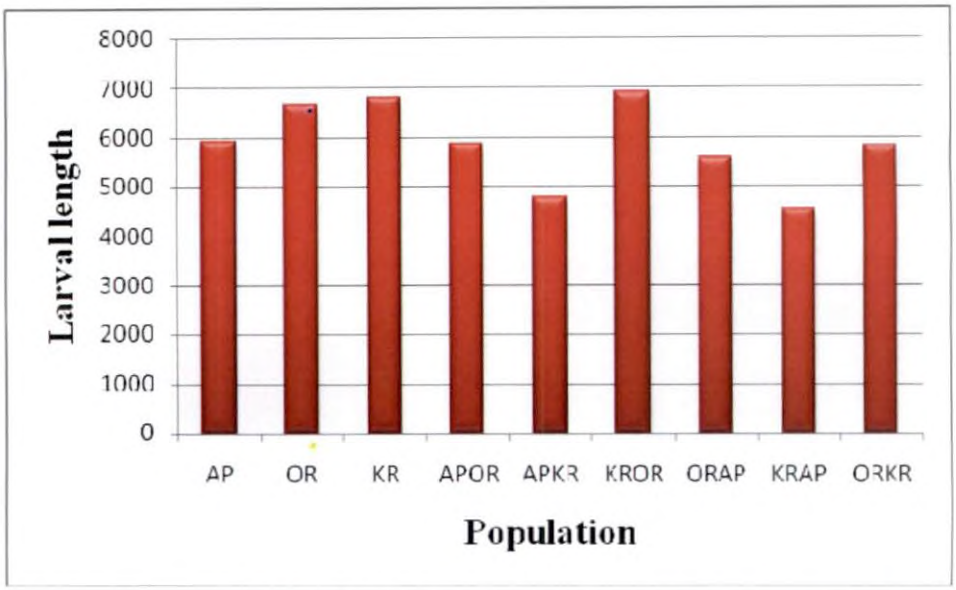

Fig.7. Mean larval length of *M.rosenbergii* for different populations.

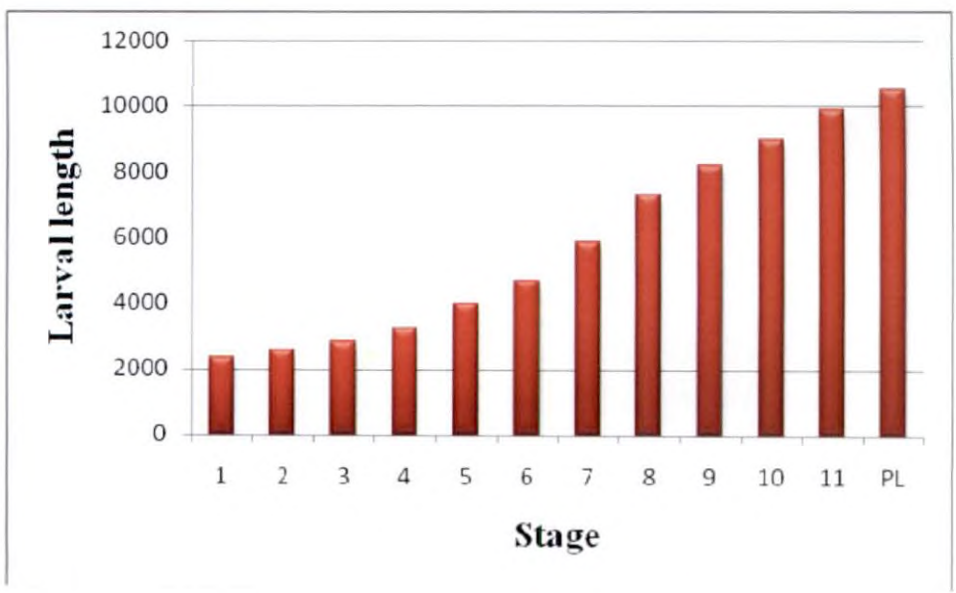

Fig.8. Length of larvae of *M.rosenbergii* at different stages

4.2.6 Larval Stress Tests

4.2.6.1 Temperature tolerance

Temperature tolerance test was carried out at the fifth and tenth stages of *M.rosenbergii* larvae of different populations and hybrids. The data on number of mortality at the fifth stage of larvae of base populations and hybrids are given in Table 39. Larvae of different populations exposed to different temperatures and mortality was estimated. Probit analysis of data gave LT_{50} values for 1 h. for different populations and hybrids (Table 40). The results are diagrammatically presented in Fig.9. It is found that among the three populations, highest LT_{50} value was for KR (37.36^0C) followed by OR (37.23^0C) and the least for AP (36.83^0C). In the case of hybrids maximum LT_{50} value was for KROR followed by ORKR. But other hybrids showed less LT_{50} than KROR, ORKR, OR and KR. LT-50 of KR was same and was on par with KROR.

Mortality of larvae (tenth stage) of different populations and hybrids of *M.rosenbergii* are given in Table 41. Probit analysis was done and the LT_{50} values are given in Table 42 and Fig. 10. From the results it is found that maximum LT_{50} value was for KROR (37.52^0C) followed by KR (37.45^0C) and OR (37.34^0C). Lowest value was obtained for APKR (36.76^0C) followed by KRAP (36.84^0C). It is also evident that tenth stage of larvae showed lesser mortality (Table 39 and 41) and higher LT_{50} (Table 40 and 42) than the larvae at the fifth stage in all the populations and hybrids. These results indicates that tenth stage of larvae were more tolerant to temperature irrespective of populations and hybrids.

Table 39 Effect of temperature on mortality of *M. rosenbergii* larvae (stage V) after one h.

Temperature (^{0}C) / Population	Mortality (no.)			
	35^{0}C	37^{0}C	39^{0}C	41^{0}C
AP	0	4.67±0.52	8.00±0.41	8.00±0.0
OR	0	3.67±0.52	7.50±0.55	8.00±0.0
KR	0	3.50±0.55	7.17±0.41	8.00±0.0
APOR	0	4.33±0.52	7.83±0.41	8.00±0.0
APKR	0	5.17±0.41	8.00±0.00	8.00±0.0
KROR	0	3.33±0.52	7.33±0.52	8.00±0.0
ORKR	0	3.50±0.55	7.67±0.52	8.00±0.0
KRAP	0	4.83±0.41	8.00±0.00	8.00±0.0
ORAP	0	4.33±0.52	7.83±0.41	8.00±0.0

Values are Mean±SD of six replications

Table 40 Lethal temperature after one h. at stage V in larvae of *M.rosenbergii*

Populations and Hybrids	LT_{50} (^{0}C)	95% Confidence Limits	
		Lower bound	Upper bound
AP	36.83	36.53	37.14
OR	37.23	36.93	37.52
KR	37.36	37.06	37.65
APOR	36.94	36.66	37.22
APKR	36.73	36.44	37.01
KROR	37.36	37.09	37.63
ORKR	37.22	36.94	37.50
KRAP	36.80	36.52	37.09
ORAP	36.98	36.70	37.26

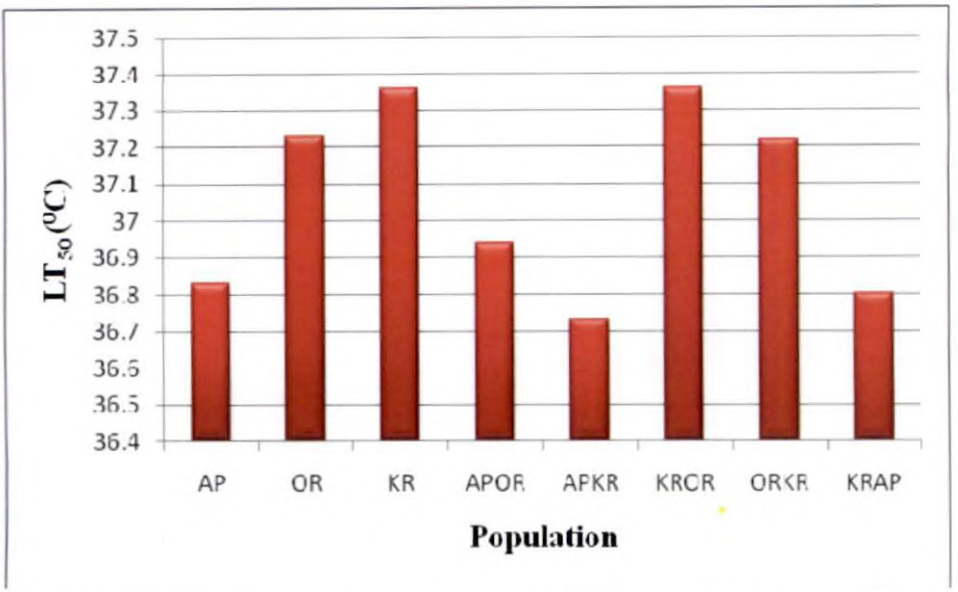

Fig.9. Lethal temperature after one h. at stage V of larvae of *M. rosenbergii*

Table 41 Effect of temperature on mortality of *M. rosenbergii* larvae (stage X) after one h.

Temperature (^{0}C) / Population	Mortality (no.)			
	35^{0}C	37^{0}C	39^{0}C	41^{0}C
AP	0	4.50±0.55	8.00±0.00	8.00±0.00
OR	0	3.50±0.55	6.33±0.52	8.00±0.00
KR	0	3.33±0.52	6.17±0.41	8.00±0.00
APOR	0	4.17±0.41	7.67±0.52	8.00±0.00
APKR	0	5.00±0.00	8.00±0.00	8.00±0.00
KROR	0	3.00±0.00	7.00±0.63	8.00±0.00
ORKR	0	3.33±0.52	7.50±0.55	8.00±0.00
KRAP	0	4.67±0.52	8.00±0.00	8.00±0.00
ORAP	0	4.17±0.41	7.67±0.52	8.00±0.00

Values are Mean±SD of six replications

Table 42 Lethal temperature after one h. at stage X in larvae of *M.rosenbergii*

Populations and Hybrids	LT_{50} (^0C)	95% Confidence Limits	
		Lower bound	Upper bound
AP	37.09	36.74	37.44
OR	37.34	37.00	37.67
KR	37.45	37.12	37.78
APOR	37.07	36.78	37.36
APKR	36.76	36.46	37.05
KROR	37.52	37.24	37.80
ORKR	37.30	37.01	37.59
KRAP	36.84	36.54	37.13
ORAP	37.07	36.78	37.36

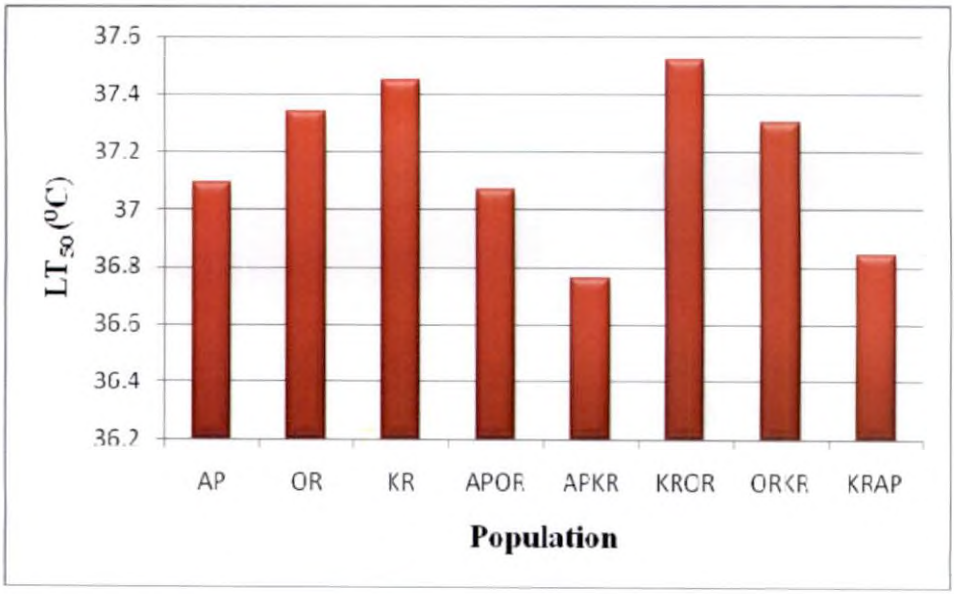

Fig. 10. Lethal temperature after one h. at stage X stage in larvae of *M. rosenbergii*

4.2.6.2 Salinity tolerance

Data pertaining to the effect of salinity on mortality no. of *M. rosenbergii* larvae (fifth stage) of populations and hybrids are given in Table 43 and Table 44. The data were subjected to probit analysis and LC_{50} values for 1 h are given in Table 45. It is diagrammatically shown in Fig. 11. The figures show that highest value was for KROR (36.04 gl^{-1}) followed by ORKR (35.13 gl^{-1}). It is also found that all the hybrids except APOR showed higher LC_{50} than parent populations. Among the populations AP showed lowest LC_{50} (29.58 gl^{-1}) followed by OR and KR.

The effect of salinity on mortality no. of *M.rosenbergii* larvae (X stage) of population and hybrids were estimated and presented in Tables 46 and 47 respectively. The results of Probit analysis of mortality data are given in Table 48 and Fig. 12. From the table it is clear that, among populations, LC_{50} of AP was lowest (32.68 gl^{-1}) followed by OR (36.12 gl^{-1}) and KR with value 36.93 gl^{-1}. In the case of hybrids, highest LC_{50} was obtained for KROR (41.31 gl^{-1}) followed by its reciprocal cross ORKR (39.18 gl^{-1}). Least value was for APKR (29.29 gl^{-1}), which is lower than parent stocks. Similarly low LC_{50} (32.42 gl^{-1}) was found in KRAP also.

Table 43 Effect of salinity on mortality of *M. rosenbergii* larvae (stage V) after one h.

Populations / Salinity (gL^{-1})	Mortality (no.)		
	Andhra Pradesh	**Orissa**	**Kerala**
12	0.00	0.00	0.00
15	0.00	0.00	0.00
20	0.00	0.00	0.00
25	0.00	0.00	0.00
30	5.17±0.41	3.33±1.03	2.67±0.82
35	7.50±0.55	6.67±0.52	6.17±0.75
50	8.00±0.00	8.00±0.00	8.00 ±0.00

Table 44 Effect of salinity on mortality of hybrid *M. rosenbergii* larvae (stage V) after one h.

Population Salinity (gl^{-1})	Mortality no.					
	APOR	APKR	KROR	ORKR	KRAP	ORAP
12	0.00	0.00	0.00	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00	0.00	0.00
25	0.00	0.00	0.00	0.00	0.00	0.00
30	0.5±0.84	0.5±0.84	0.00	0.00	0.00	0.00
35	8.00±00	8.00±00	3.67±0.52	4.5±0.55	8.00±00	8.00±00
50	8.00±00	8.00±00	8.00±00	8.00±00	8.00±00	8.00±00

Values are mean±SD of six replications

Table 45 Lethal concentration of salinity after one h. at stage V in larvae of *M.rosenbergii*

Populations and Hybrids	LC_{50} (gl^{-1})	95% Confidence Limits	
		Lower bound	Upper bound
AP	29.58	28.77	30.39
OR	30.75	29.93	31.59
KR	31.92	31.10	32.77
APOR	32.56	31.67	33.49
APKR	31.25	30.40	32.12
KROR	36.04	34.98	37.23
ORKR	35.13	34.14	36.21
KRAP	32.34	31.45	33.27
ORAP	32.54	31.64	33.47

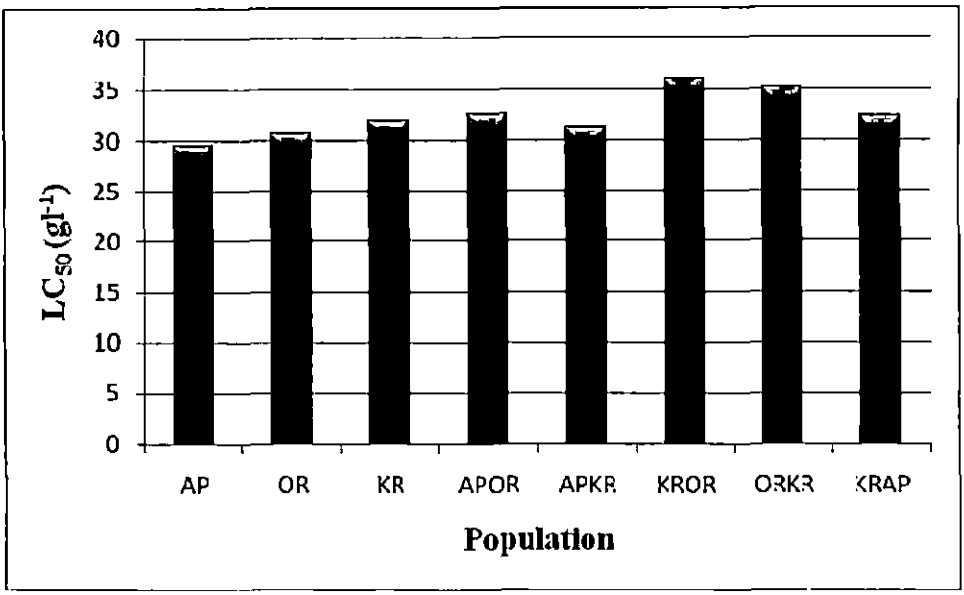

Fig. 11. Lethal concentration of salinity for one h. at stage V in larvae of *M. rosenbergii*

Table 46 Effect of salinity on mortality of *M. rosenbergii* larvae (stage X) after one h.

Populations / Salinity (gL^{-1})	Mortality (no.)		
	Andhra Pradesh	Orissa	Kerala
12	0.00	0.00	0.00
15	0.00	0.00	0.00
20	0.00	0.00	0.00
25	0.00	0.00	0.00
30	0.00	0.00	0.00
35	7.50±0.55	3.33±0.52	2.67±0.52
50	8.00±0.00	8.00±0.00	8.00±0.00

Values are mean±SD of six replications

Table 47 Effect of salinity on mortality of *M. rosenbergii* larvae of hybrids (stage X) after one h.

Population / Salinity (gl^{-1})	Mortality no.					
	APOR	APKR	KROR	ORKR	KRAP	ORAP
12	0.00	0.00	0.00	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00	0.00	0.00
25	0.00	0.00	0.00	0.00	0.00	0.00
30	0.00	4.33±0.52	0.00	0.17±0.41	3.33±0.52	1.83±0.41
35	1.5±0.55	8.00±0.00	0.00	1.33±0.52	4.83±0.41	2.67±0.52
50	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00

Values are mean±SD of six replications

Table 48 Lethal concentration of salinity after one h. at stage X in larvae of *M.rosenbergii*

Populations and Hybrids	LC_{50} (gl^{-1})	95% Confidence Limits	
		Lower bound	Upper bound
AP	32.68	31.784	33.624
OR	36.12	35.044	37.482
KR	36.93	35.751	38.499
APOR	39.01	37.313	40.842
APKR	29.29	28.266	30.347
KROR	41.31	39.327	43.434
ORKR	39.18	37.479	41.024
KRAP	32.42	31.321	33.573
ORAP	35.44	34.144	36.850

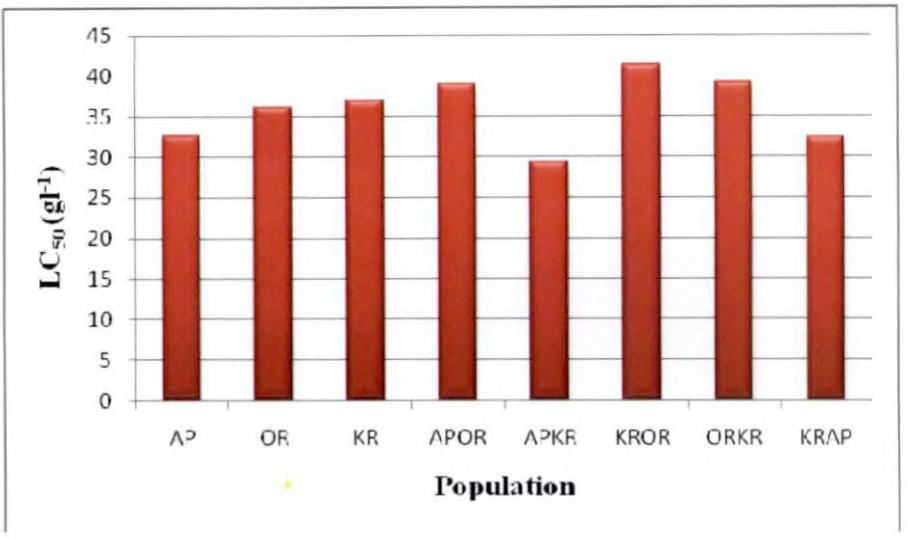

Fig. 12. Lethal concentration of salinity after one h. at X stage of larvae of *M.rosenbergii*

4.2.6.3 Formaldehyde tolerance

Data pertaining to the effect of formaldehyde on mortality no.of *M.rosenbergii* larvae (fifth stage) of populations and hybrids after 3 h. are given in Tables 49 and 50. Probit analysis was done and the LC_{50} values are given in Table 51. Graphical presentation of the same is shown in Fig. 13. From the LC_{50} values it was found that the highest tolerance was for hybrid KROR (286.65 mg/l) followed by ORKR (273.48 mg/l). Minimum LC_{50} was shown by APKR (184.17 mg/l) followed by KRAP (184.55). Among parent populations highest LC_{50} value was obtained for KR (265.97 mg/l) followed by OR (216.46 mg/l) and AP (196.30 mg/l).

Formaldehyde tolerance of larvae (stage X) of populations and hybrids of *M.rosenbergii* were estimated and the results are given in Table 52 and 53. Probit analysis was done and the LC_{50} values of populations and hybrids are summarised in Table 54 and Fig. 14. Maximum value was for KROR (361.88 mg/l) and minimum for KRAP (185.22 mg/l). Among present stocks, minimum value was for AP followed by OR and KR. Larvae of tenth stage showed higher LC_{50} values than that of fifth stage in all cases.

Table 49 Effect of formaldehyde on mortality of *M. rosenbergii* larvae (stage V) after three h.

Population / Formaldehyde (mgl^{-1})	Mortality (no)		
	Andhra Pradesh	Orissa	Kerala
50	0.00	0.00	0.00
100	0.00	0.00	0.00
150	0.67±0.52	0.00	0.00
250	7.17±0.41	6.67±0.52	2.33±0.52
350	8.00±0.00	8.00±0.00	8.00±0.00

Values are mean±SD of six replications

Table 50 Effect of formaldehyde on mortality of hybrid *M. rosenbergii* larvae (stage V) after three h.

Formaldehye (mgl^{-1})	Mortality no.					
	APOR	APKR	KROR	ORKR	KRAP	ORAP
50	0.00	0.00	0.00	0.00	0.00	0.00
100	0.00	0.00	0.00	0.00	0.00	0.00
150	0.00	1.17±0.41	0.00	0.00	1.33±0.52	0.33±0.52
250	3.50±0.55	7.50±0.55	1.00±0	1.67±0.82	7.33±0.52	3.67±0.52
350	8.00±0.00	8.00±0.00	7.67±0.52	8.00±0.00	8.00± 0.00	8.00±0.00

Values are mean±SD of six replications

Table 51 Lethal concentration of formaldehyde after three h. at stage V in larvae of *M.rosenbergii*

Populations and Hybrids	LC_{50} (mgl^{-1})	95% Confidence Limits	
		Lower bound	Upper bound
AP	196.30	184.28	208.93
OR	216.46	202.49	230.08
KR	265.97	252.47	280.02
APOR	252.49	238.69	266.77
APKR	184.17	172.90	196.34
KROR	286.65	271.55	302.57
ORKR	273.48	258.90	288.78
KRAP	184.55	173.48	196.47
ORAP	243.40	230.25	257.09

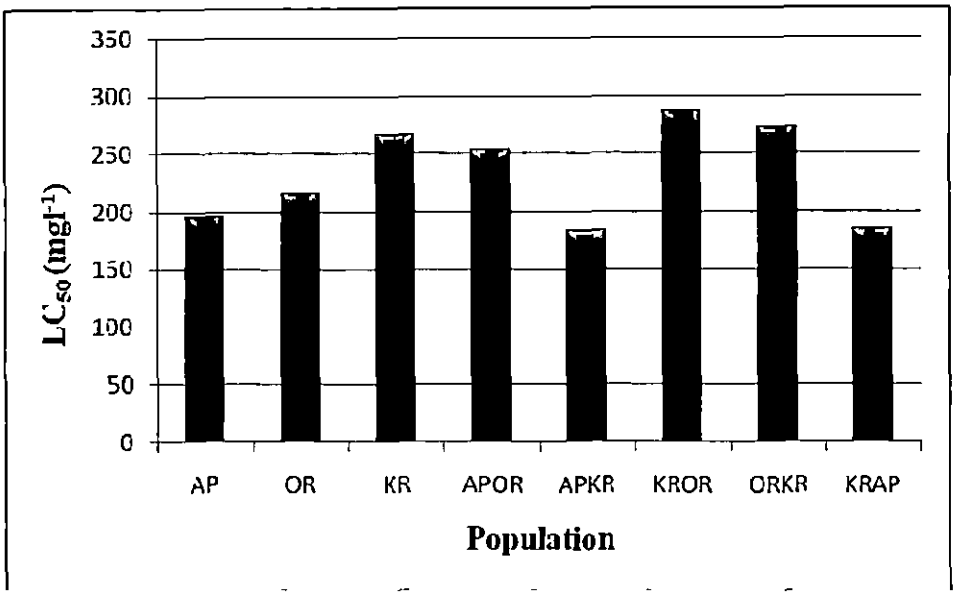

Fig 13. Lethal concentration of formaldehyde for three h. in larvae at stage V of *M.rosenbergii*

Table 52 Effect of formaldehyde on mortality of *M. rosenbergii* larvae (X stage) after three h.

Formaldehyde (mgL^{-1})	Mortality (no)		
	Andhra Pradesh	Orissa	Kerala
50	0.00	0.00	0.00
100	0.00	0.00	0.00
150	0.17±0.41	0.00	0.00
250	1.33±0.52	0.33±0.52	0.17±0.41
350	7.17±0.75	6.67±0.82	4.5±0.55

Values are mean±SD of six replications

Table 53 Effect of formaldehyde on mortality of larvae of hybrid (stage X) after three h.

Population / Formaldehye (mgL^{-1})	Mortality (no)					
	APOR	APKR	KROR	ORKR	KRAP	ORAP
50	0	0	0	0	0	0
100	0	0	0	0	0	0
150	0	0.83±0.75	0	0.17±0.41	1.33±0.52	0.33±0.52
250	0.83±0.98	7±0	0	1.33±0.82	7.33±0.52	1.67±0.52
350	4.67±0.52	7.83±0.41	4.33±0.52	5±0.63	8±0	4.83±0.41

Values are mean±SD of six replications

Table 54 Lethal concentration of formaldehyde after three h. at stage X in larvae of *M.rosenbergii*

Populations and Hybrids	LC_{50} (mgl^{-1})	95% Confidence Limits	
		Lower bound	Upper bound
AP	284.51	127.299	690.192
OR	309.80	136.360	784.393
KR	342.61	190.122	1345.279
APOR	336.48	310.110	366.417
APKR	197.86	181.677	215.244
KROR	361.88	331.538	397.012
ORKR	316.72	292.881	343.410
KRAP	185.22	170.013	201.771
ORAP	309.24	286.492	334.625

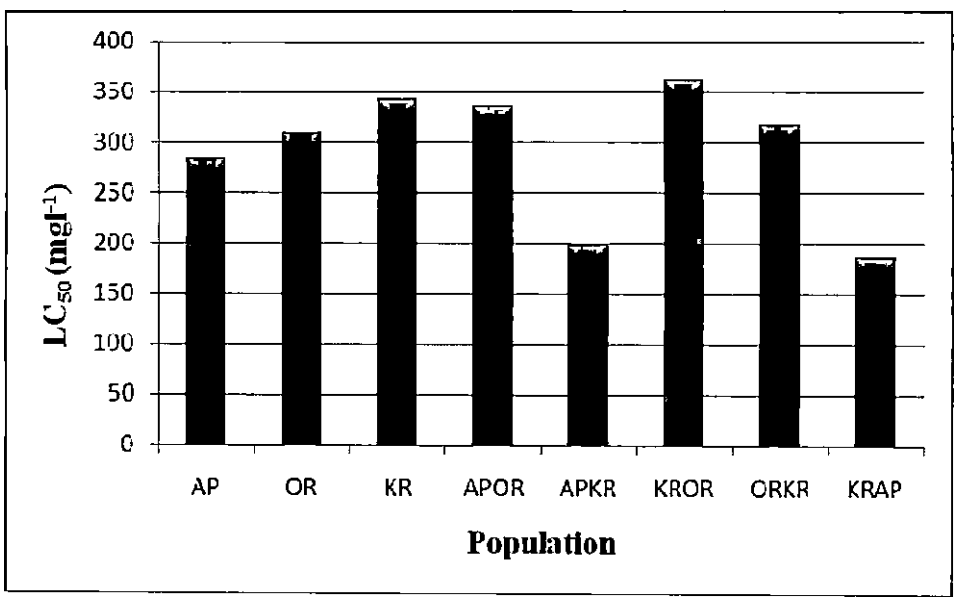

Fig 14. Lethal concentration of formaldehyde for three h. at X stage of larvae of *M.rosenbergii*

4.2.6.4 Ammonia tolerance

Data pertaining to the mortality no. of larval stage (V) of different populations and hybrids of *M.rosenbergii* in different concentrations of ammonia are given in Table 55 and 56. LC_{50} value obtained from probit analysis of populations and hybrids are given in Table 57 and its graphical representation in Figure 15. Values of LC_{50} of ammonia showed variations among populations and hybrids. Maximum LC_{50} value was found for KR (140.25 mg/l) followed by KROR (139.21 mg/l). Lowest ammonia tolerance was exhibited by KRAP with 124.71 mg/l followed by its reciprocal cross APKR (127.05 mg/l).

Mortality no. of different concentrations of ammonia for different populations and hybrids of *M.rosenbergii* larvae (tenth stage) for six h. were estimated and the results are given in Table 58 and 59. Results of Probit analysis for different populations and hybrids are shown in Table 60 and LC_{50} values are diagrammatically presented in Fig. 16. It is clear from the table that higher tolerance to ammonia was for KROR with LC_{50} value 132.66 mg/l, followed by KR with 132.29 mg/l. Lowest LC_{50} value was shown by KRAP (119.86 mg/l) followed by AP (120.72 mg/l). LC_{50} values of fifth and tenth stage showed a marked difference in tolerance level of ammonia. Mortality no. at fifth stage was less compared to tenth stage for all concentrations.

Table 55 Effect of ammonia on mortality of *M. rosenbergii* larvae (stage V) after six h (pH 8.3)

Total Ammonia concentrations (mg/l)	Mortality (no)		
	Andhra Pradesh (1)	Orissa (2)	Kerala (3)
100	0.00	0.00	0.00
110	1.00±0.00	0.67±0.52	0.00
120	2.00±0.00	1.33±0.52	1.00±0.00
130	3.00±0.00	2.17±0.41	2.00±0.00
140	5.67±0.52	3.00±0.00	2.33±0.52
150	7.83±0.41	6.00±0.00	6.00±0.00
160	8.00±0.00	8.00±0.00	8.00±0.00

Values are mean±SD of six replications

Table 56 Effect of ammonia on mortality of hybrid *M. rosenbergii* larvae (stage V) after six h (pH 8.3).

Total NH3 mg/l	Mortality (no.)					
	APOR	APKR	KROR	ORKR	KRAP	ORAP
100	0.00	0.00	0.00	0.00	0.00	0.00
110	1.00±0.00	1.33±0.52	0.00	0.33±0.52	1.67±0.52	1.17±0.41
120	2.00±0.00	2.17±0.41	1.00±0.00	1.5±0.55	2.33±0.52	2.33±0.52
130	2.17±0.41	4.00±0	2.00±0.00	2.67±0.52	4.5±0.55	2.5±0.54
140	5.00±0.00	6.83±0.41	3.17±0.41	3.83±0.41	7.33±0.52	5.33±.52
150	7.00±0.00	7.33±0.52	6.00±0.00	7.17±0.41	7.67±0.52	7.5±0.55
160	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00

Values are mean±SD of six replications

Table 57 Lethal concentration of ammoniae after six h. at stage V in larvae of *M.rosenbergii*

Populations and Hybrids	LC_{50} (mg/l)	95% Confidence Limits	
		Lower bound	Upper bound
AP	129.61	127.07	132.19
OR	137.45	134.83	140.17
KR	140.25	137.50	143.10
APOR	132.41	129.89	134.99
APKR	127.05	124.58	129.56
KROR	139.21	136. 50	141.99
ORKR	134.71	132.12	137.37
KRAP	124.71	122.26	127.21
ORAP	130.32	127.83	132.86

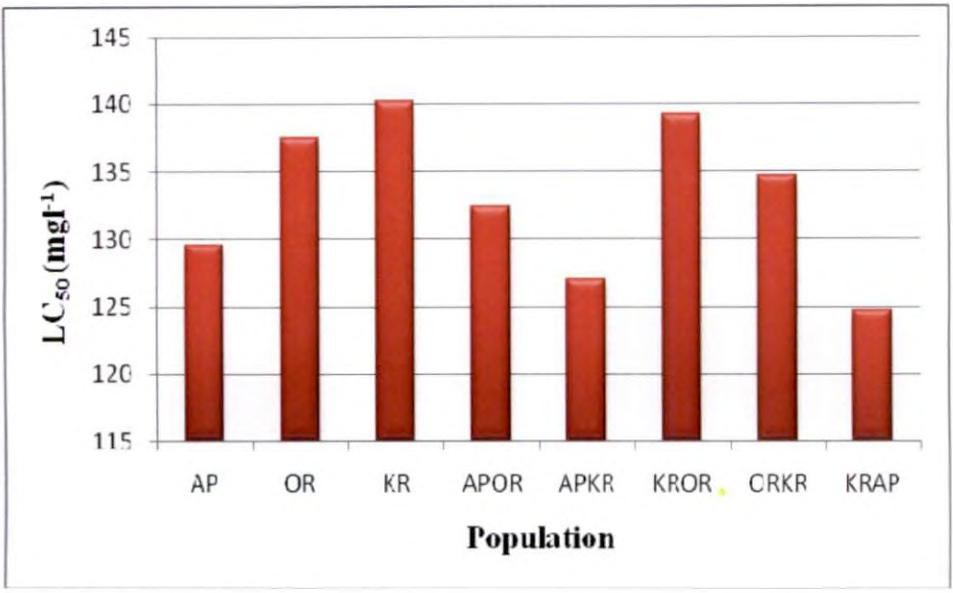

Fig. 15. Lethal concentration of ammonia for six h. on V stage of larvae of *M.rosenbergi*

Table 58 Effect of ammonia on mortality of *M. rosenbergii* larvae (stage X) after six h (pH 8.3).

Concentrations of Ammonia (mg/l)	Mortality (no)		
	Andhra Pradesh	Orissa	Kerala
100	0.00	0.00	0.00
110	2.00±0.00	1.17±0.41	1.00±0.00
120	4.17±0.41	2.00±0.00	1.17±0.41
130	6.17±0.41	4.00±0.00	3.17±0.41
140	7.00±0.00	5.83±0.41	5.00±0.00
150	8.00±0.00	8.00±0.00	8.00±0.00

Values are mean±SD of six replications

Table 59 Effect of ammonia on mortality of hybrid *M. rosenbergii* larvae (stage X) after six h (pH 8.3).

Total NH3 mg/l	Mortality (no.)					
	APOR	APKR	KROR	ORKR	KRAP	ORAP
100	0.00	0.00	0.00	0.00	0.00	0.00
110	1.33±0.52	2.17±0.41	0.83±0.41	1.00±0.00	2.67±0.52	1.50±0.55
120	3.00±0.00	4.17±0.41	1.17±0.41	1.50±0.55	4.50±0.55	3.50±0.55
130	4.00±0.00	4.00±0.00	3.00±0.00	3.67±0.52	4.67±0.52	4.33±0.52
140	5.67±5.67	7.33±0.52	4.33±0.52	4.67±0.52	7.67±0.52	5.67±0.52
150	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00

Values are mean±SD of six replications

Table 60 Lethal concentration of ammonia after six h. at stage X in larvae of *M.rosenbergii*

Populations and Hybrids	LC_{50} (mg/l)	95% Confidence Limits	
		Lower bound	Upper bound
AP	120.72	118.45	123.04
OR	127.50	125.22	129.88
KR	132.29	129.60	135.02
APOR	126.79	124.22	129.42
APKR	121.90	119.39	124.44
KROR	132.66	129.91	135.49
ORKR	130.86	128.18	133.62
KRAP	119.86	117.37	122.38
ORAP	125.58	123.04	128.17

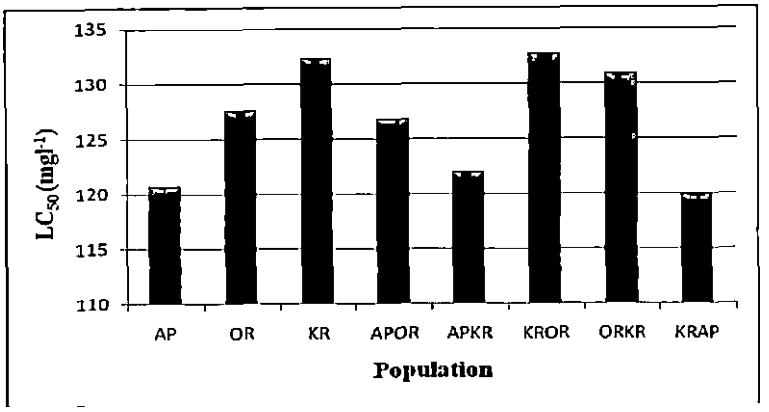

Fig. 16 Lethal concentration of ammonia for six h. at X stage of larvae of *M.rosenbergii*

4.3 COMPARISON OF ECONOMICALLY IMPORTANT TRAITS

4.3.1 Disease Resistance

4.3.1.1 Viral challenge study

The mean mortality no. of different populations of *M. rosenbergii* juveniles in challenge study with extract of prawns infected with white tail virus are given in Table 61. Minimum mortality (3 no) was observed for Kerala (KR) population and its hybrid with Orissa (KROR). Maximum mortality (4 no) was for hybrid APKR. The mortality at different concentrations of viral inocula is also given in Table 61. Graphical presentation of mortality of different populations and hybrids in challenge study is given Fig 17. Similarly, the mortality no. for different concentrations (treatments) is shown Fig. 18. From the table it is clear that there was no mortality for T_0 (control) and T_1 (100µl inoculums). Maximum mortality (8 no) was occurred for T_5 (200 µl inoculum). The Analysis of Variance (Table 62) of data on mortality of prawns showed that the mean mortality was significantly different among different populations and among treatments ($p<.001$). The mortality rate exhibited by KR and KROR was significantly lower than all other populations/ hybrids ($p<0.05$). The mortality reported in all other populations were also significantly different from each other ($p<0.05$). Statistical analysis also revealed significant interaction between populations and treatments.

Table: 61 Mortality no. of different stocks of juveniles with different treatments in challenge study with white tail virus for forty eight h

Treatments / Populations	Mortality rate (No) (Mean±SD)						Population mean
	T_0 Control	T_1 100 µl	T_2 125 µl.	T_3 150 µl	T_4 175µl	T_5 200 µl	
AP	0^{l}	0^{j}	2.84^{l}±0.41	6.0^{p} ±0 0	7.5^{s} ±0.55	8.0^{t}±0	4.06^{d}±3.62
OR	0^{j}	0^{j}	2.0^{k} ±0.0	5.0^{o}±0.0	6.67^{q}±0.52	8.0^{t} ±0	3.61^{b}±3.44
KR	0^{j}	0^{j}	2.0^{k} ±0.0	4.0^{n} ±0.0	6.0^{p}± 0.0	8.0^{t}±0.0	3.33^{a}±3.27
APOR	0^{j}	0^{j}	3.0^{m}±0.0	5.167^{o}±0.41	7.0^{r}± 0.0	8.0^{t}±0.0	3.86^{c}±3.44
APKR	0^{j}	0^{j}	3.167^{m}±0.41	6.0^{p}±0.0	7.33^{s} ±0.52	8.0^{t}±0.0	4.08^{d}±3.57
KROR	0^{j}	0^{j}	2.0^{k}±0.0	4.0^{n}±0.0	6.0^{p} ±0.0	8.0^{t}±0.0	3.33^{a}±3.27
Treatment mean	0^{e}	0^{e}	2.50^{f} ±0.56	5.03^{g} ±0.89	6.75^{h} ±0.65	8^{i} ±0.0	

Values are mean±SD of six replications
Mean values with same or common superscript do not differ significantly

Table 62 ANOVA of mortality no. of different stocks of juveniles with different treatments in challenge study with white tail virus for forty-eight h.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability (p).
Replication	5	0.14	0.03	0.78	
Population	5	19.48	3.90	109.28*	<.001
Treatment	5	1632.89	326.58	9162.26*	<.001
Population* Treatment	25	24.05	0.96	26.99*	<.001
Residual	175	6.24	0.04		
Total	215	1682.79			

*Significant (p<0.001)

(Data subjected to square root transformation)

LSD (0.05) for Population mean: 0.0878

LSD (0.05) for treatment mean: 0.0878

LSD (0.05) for Population*Treatment: 0.2151

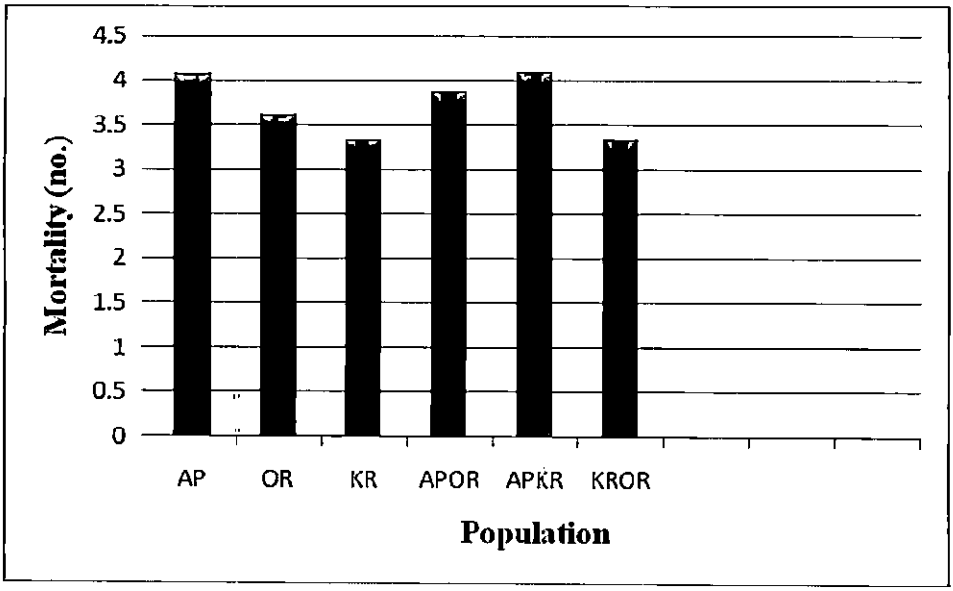

Fig 17 Mortality of different stocks of *M. rosenbergii* juveniles in challenge study with white tail virus for forty-eight h.

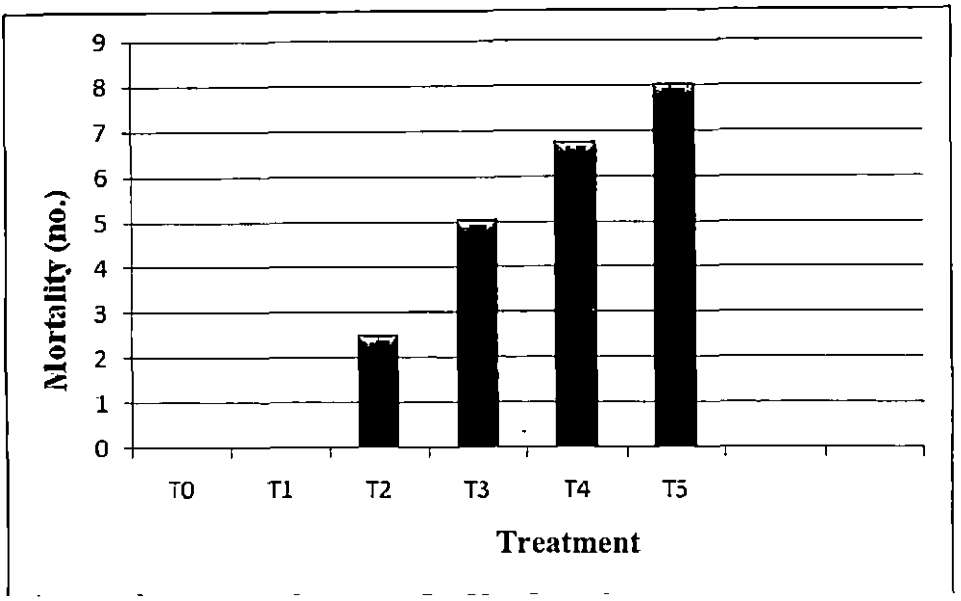

Fig 18 Mortality of *M. rosenbergii* juveniles in different treatments with white tail virus for forty-eight h.

4.3.1.2 Bacterial challenge study

The mean mortality of six populations of juvenile *M.rosenbergii* in six treatments of different concentrations of *Enterococcus aerogenes* bacterial inocula (challenge study) is given in Table 63. The table shows that KR and KROR showed minimum mortality (3 no.). Maximum mortality (4 no.) was reported in APKR followed by APOR, AP and OR respectively. It is also found that no mortality occurred in T_0 (control) and T_1 and the mortality rates showed an increasing trend from T_0 to T_5. Mortality rate of different populations and treatments are shown in Figs. 19 and 20 respectively.

Analysis of variance (Table 64) of mortality of prawns showed that there is significant variation among populations, among treatments and in their interaction ($p<0.001$). Comparison using LSD revealed that the two populations having minimum mortality (KR and KROR) were similar, but significantly different from other populations. All other populations were also significantly different from each other. The lowest mean mortality rate of treatments recorded in T_0 and T_1 were on par with each other and different significantly from other treatments.

Table: 63 Mortality of different stocks of *M. rosenbergii* juveniles with different treatments in bacterial challenge study for forty-eight h.

Treatment / Population	Mortality rate (no.) (Mean±SD)						Population mean
	T_0 Control	T_1 (10^{-4})	T_2 (10^{-3})	T_3 (10^{-2})	T_4 (10^{-1})	T_5 (Stock soln.)	
AP	0^{k}	0^{k}	2^{l} ±00	3^{m} ±00	8^{s} ±00	8^{s} ±00	3.50^{e} ±3.67
OR	0^{k}	0^{k}	0^{k}	4^{n} ±00	7^{r} ±00	8^{s} ±00	3.17^{b} ±3.71
KR	0^{k}	0^{k}	0^{k}	4^{n} ±00	6^{p} ±00	8^{s} ±00	3.00^{a} ±3.52
APOR	0^{k}	0^{k}	2^{l} ±00	5^{o} ±00	7^{r} ±00	8^{s} ±00	3.67^{d} ±3.50
APKR	0^{k}	0^{k}	3^{m} ±00	6.17^{q}±0.41	8^{s} ±00	8^{s} ±00	4.20^{e} ±3.73
KROR	0^{k}	0^{k}	0^{k}	4^{n} ±00	6^{p} ±00	8^{s} ±00	3.00^{a} ±3.52
Treatment mean	0^{f}	0^{f}	1.17^{g}±1.33	4.36^{h}±1.09	7.00^{i} ±0.89	8.00^{j} ±00	

Values are mean±SD of six replications
Mean values with same or common superscript do not differ significantly

Table 64 ANOVA of mortality of different stocks of juveniles with different treatments in bacterial challenge study for forty-eight h.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability p.
Replication	5	0.02	0.05	1.00	
Population	5	27.22	75.45	1204.07*	<.001
Treatment	5	1.81	361	79834.65*	<.001
Population* Treatment	25	59.85	2.39	529.42*	<.001
Residual	175	0.79	0.05		
Total	215	1893			

*Significant ($p<0.001$)
(Data subjected to square root transformation)

L.S.D (0.05) for population: 0.03128

L.S.D (0.05) for treatment: 0.03128

L.S.D (0.05) for population *treatment: 0.07662

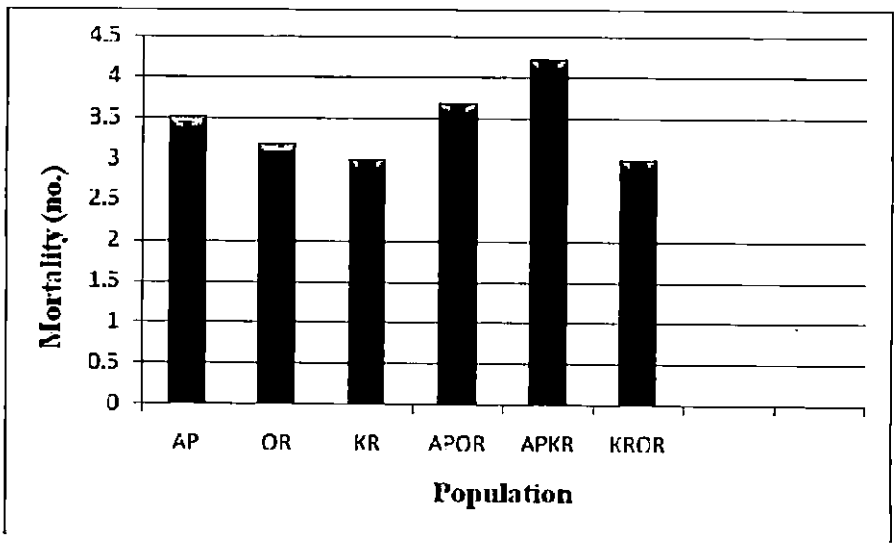

Fig 19 Mortality rate of different stocks of *M. rosenbergii* juveniles in challenge study with bacteria for forty-eight h.

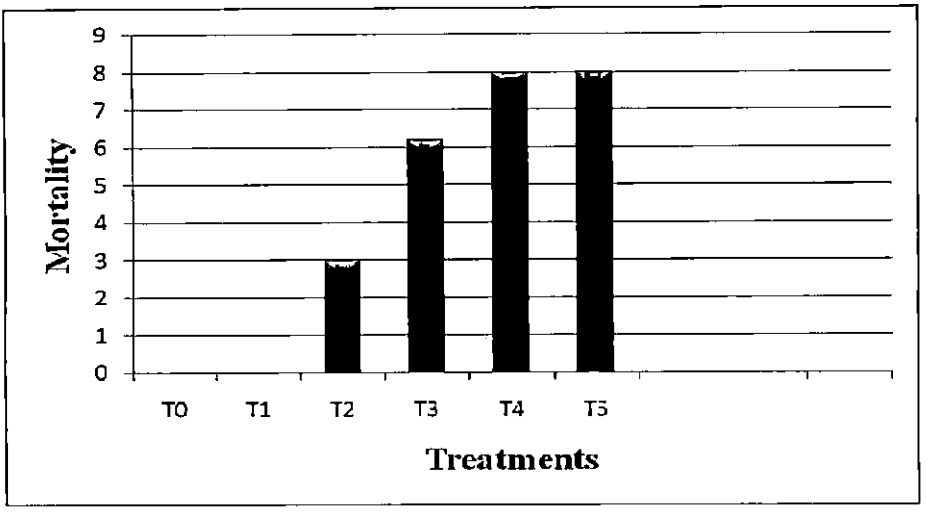

Fig 20 Mortality rate of *M. rosenbergii* juveniles in challenge study with different concentrations of bacteria for forty-eight h.

4.3.1.3 Prophenoloxidase activity (PPO)

Prophenoloxidase activity of various populations and their hybrids in control and bacterial challenged prawns after six hours were evaluated and recorded (Table 65). Graphical presentation of PPO in populations and hybrids are shown in Fig. 21. From the table it is clear that PPO was maximum for population KROR (11.00eu) followed by KR (10.49eu) and least for APKR (7.06eu). Analysis of Variance (Table. 66) of data on PPO showed overall significance ($p<0.001$) in populations, treatments, time and their interactions as per LSD test (Table 65). All the population means were significantly different each other.

Mean PPO of control and T_1 (bacteria inoculated prawns) are also given in the Table 65. From the table it is clear that PPO value was higher for T_1 (14.44eu) compared to control (4.12eu). From the Analysis of variance (Table 66), it is evident that the treatment means were significantly different ($p<0.001$). PPO after one minute and three minutes in control and challenged prawns were also recorded (Table. 65). PPO values for one minute were lower than those of three minutes, both in control and T_1 for all the populations.

It is also evident from the Table 65 that PPO activity was lesser in control than T_1 for all the populations. In the case of challenged prawns for one minute, the mean PPO values ranged from 10.73 eu (APKR) to 16.79 eu (KROR). The corresponding values at three minutes were 11.21 eu (APKR) and 17.86 eu (KROR). These results show that the lower PPO activity occurred in APKR and highest in KROR at both time periods. Statistical analysis showed that means for T_1 were significantly different from each other except between AP at three minutes and APOR at one minute ($p<0.05$).

Table 65 Prophenoloxidase activity of different populations and hybrids of juveniles challenged with bacteria after six h.

Treatment / Population	Prophenoloxidase activity (Enzyme unit)				Population Mean
	Control(C)		Challenged prawns(T_1)		
	Time		Time		
	1 minute	3 minutes	1 minute	3 minutes	
AP	3.83 ±0.02	3.94 ±0.01	12.84^{i} ± 0.01	13.03^{j} ± 0.02	8.41^{b} ±5.23
OR	4.28± 0.01	4.38 ± 0.01	15.73^{l} ± 0.01	16.03^{m} ± 0.01	10.10^{d} ±6.67
KR	4.56 ±0.03	4.63 ± 0.02	16.32^{n} ± 0.53	16.43^{o} ± 0.01	10.49^{e}±6.8
APOR	3.96 ± 0.02	4.22 ± 0.01	12.95^{j} ±0.01	13.42^{k}± 0.01	8.65^{c} ±5.26
APKR	3.08±0.79	3.22±0.09	10.73^{g}± 3.68	11.21^{h} ±3.101	7.06^{a} ±4.52
KROR	4.60 ±0.01	4.79 ± 0.01	16.79^{p}± 0.01	17.86^{q}± 0.014	11.01^{f} ±7.31
Treatment Mean	4.12^{r}±0.54		14.44^{s}±2.35		

Enzyme unit*- the amount of enzyme giving an increase in .001 absorbance at 490nm

Values are mean±SD of six replications

Mean values with same or common superscript do not differ significantly.

Table 66 ANOVA of prophenoloxidase activity of different populations and hybrids of juveniles *M.rosenbergoii* challenged with bacteria after six h.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	probability
Replication	5	0.07	0.01	1.26	
Population and hybrids	5	269.29	53.86	4596.67*	<.001
Treatment	1	3833.26	3833.26	3.272*	<.001
Time	1	3.04	3.04	259.39*	<.001
Population*Treatment	5	110.24	22.05	1881.79*	<.001
Population*Time	5	1.14	0.23	19.38*	<.001
Treatment*Time	1	0.77	0.77	65.35*	<.001
Pop*Treat*Time	5	0.70	0.14	11.89*	<.001
Residual	115	1.35	0.01		
Total	143	4219.85			

*Significant ($p<0.001$)

LSD (0.05) for population means: 0.06189

LSD (0.05) for treatment means: 0.03573

LSD (0.05) for population*treatment interaction: 0.08753

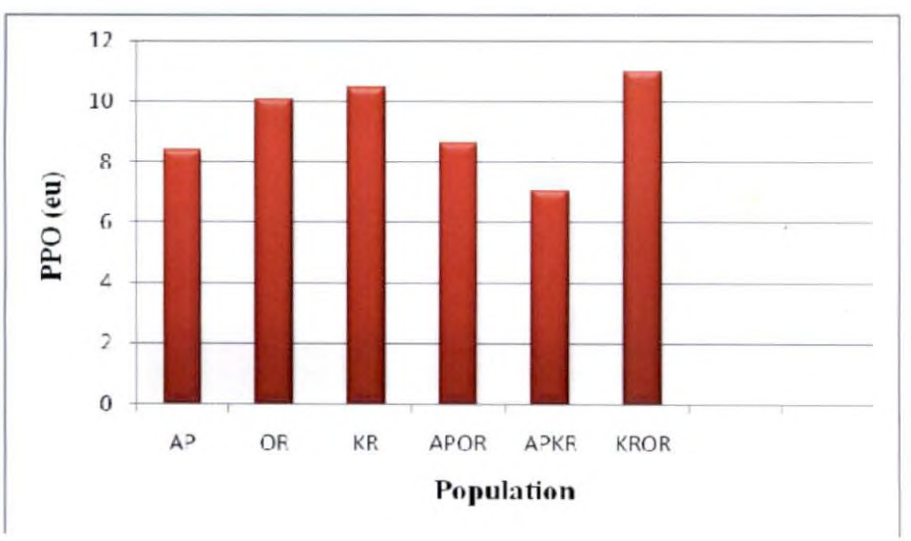

Fig 21 Prophenoloxidase activity of different populations and hybrids of *M. rosenbergii*

4.3.1.4 Total haemocyte count (THC)

Total haemocyte count of untreated (control) and bacteria challenged juveniles of *M. rosenbergii* after six hours were determined. The mean haemocyte counts of different populations and treatments are given in Table 67 and its graphical presentation is shown in Figures 22 and 23. The table shows that the highest THC was for Kerala population (184.92 × 10^5 no/ml) followed by KROR hybrid (183.63× 10^5 no/ml). Lowest count was for APKR (115.16× 10^5 no/ml) followed by AP, APOR and OR. Analysis of Variance (Table 68) revealed that the data on haemocyte count showed overall significance ($p<0.001$) in populations, treatments and their interaction. The population means were significantly different among all populations except between KROR and KR, as per LSD test (Table 67).

Mean count of control and T_1 (bacteria inoculated prawns) are also given in the Table 49. From the table it is found that the haemocyte count was higher for control (222.74×10^5) compared to T_1 (81.51× 10^5). Analysis of variance (Table 68) revealed that there is significant variation between control and T_1 ($p<0.001$).

Haemocyte count of T_1 were significantly lower than control for all populations ($p<0.05$). KR and KROR showed higher counts, which were on par with each other in both control and T_1. APKR, AP and APOR reported lesser counts in both control and challenged prawns.

Table 67 Total haemocyte count of different populations and hybrids of juveniles in bacterial challenge study after six h.

Treatment / Populations	Haemocyte count (no./ml) (mean±SD)		Population mean
	Control (C)	Challenged prawns(T1)	
AP	$187.83^{l} \times 10^5 \pm 7.31$	$76.62^{hi} \times 10^5 \pm 6.44$	$132.23^{b} \times 10^5 \pm 78.64$
OR	$231.50^{n} \times 10^5 \pm 11.22$	$83.33^{ji} \times 10^5 \pm 4.97$	$157.42^{d} \times 10^5 \pm 104.77$
KR	$279.17^{lo} \times 10^5 \pm 9.87$	$90.67^{j} \times 10^5 \pm 6.22$	$184.92^{e} \times 10^5 \pm 133.30$
APOR	$201.92^{m} \times 10^5 \pm 17.11$	$76.90^{hi} \times 10^5 \pm 3.03$	$139.41^{c} \times 10^5 \pm 88.40$
APKR	$157.77^{k} \times 10^5 \pm 6.98$	$72.55^{h} \times 10^5 \pm 3.22$	$115.16^{a} \times 10^5 \pm 60.26$
KROR	$278.25^{o} \times 10^5 \pm 2.89$	$89.00^{j} \times 10^5 \pm 4.69$	$183.63^{e} \times 10^5 \pm 133.82$
Treatment Mean	$222.74^{g} \times 10^5 \pm 49.43$	$81.51^{f} \times 10^5 \pm 7.33$	

Values are mean±SD of seven replications

Mean values with same superscript do not differ significantly

Table 68 ANOVA of total haemocyte count of three stocks and hybrids of juveniles in bacterial challenge study after six h.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability (p).
Replication	5	286.80	57.36	0.88	
Population and hybrids	5	48237.64	9647.53	148.31*	<.001
Treatment	1	359015.13	359015.13	5519.09*	<.001
Population.*Treatment	5	26668.97	5333.79	82.00 *	<.001
Residual	55	3577.73	65.05		
Total	71	437786.28			

*Significant ($p<0.001$)

LSD (0.05) for population means: 6.599

LSD (0.05) for treatment means: 3.810

LSD (0.05) for population*treatment: 9.332

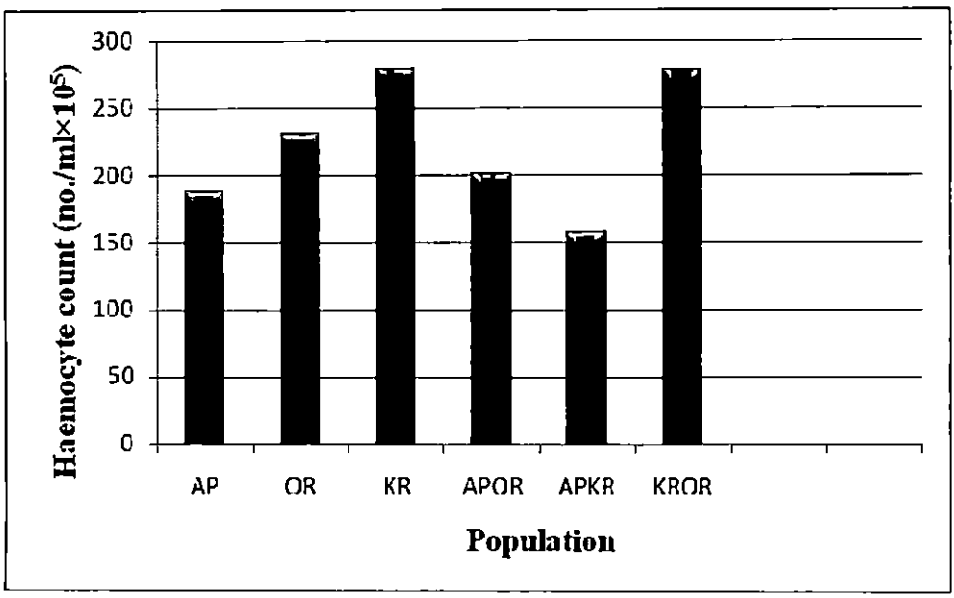

Fig 22 Total haemocyte count of three stocks of juvenile *M.rosenbergii* and hybrids in control after six h.

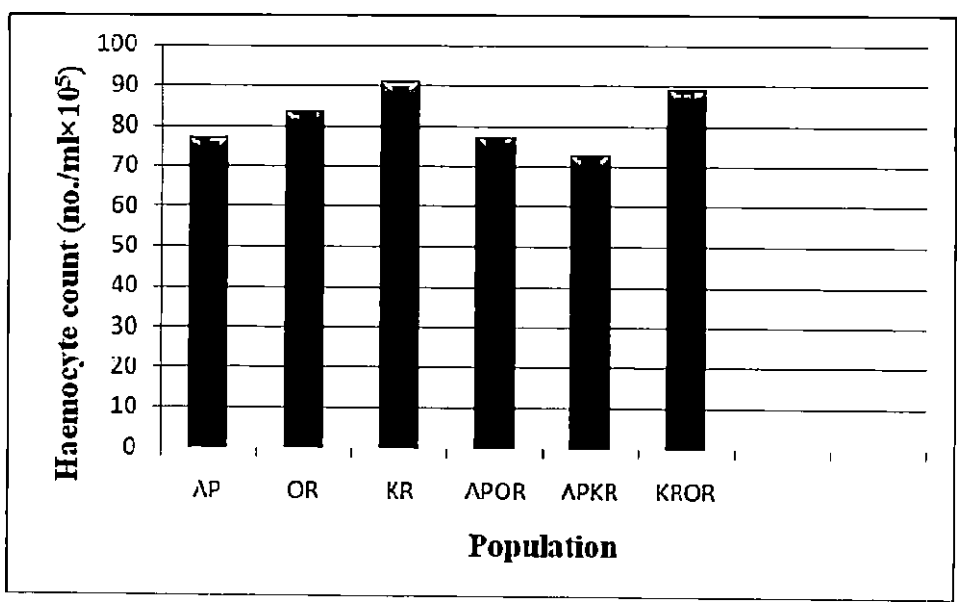

Fig 23 Total haemocyte count of three stocks of juvenile *M.rosenbergii* and hybrids in bacterial challenge study after six h.

4.3.2 Carcass composition

The carcass composition of different stocks of *M.rosenbergii* juveniles was estimated and the results are given in Table 69. Analysis of variance of the data (Table 70-74) was carried out for body composition of different populations. From the ANOVA tables it is clear that there were no significant variation among the populations in respect of moisture, protein, lipid, ash and carbohydrate content of body.

Table 69 Carcass composition (Mean±SD) of different stocks of juvenile *M.rosenbergii*

Population	Parameters				
	Moisture (%)	Crude Protein (%)	Crude lipid (%)	Crude ash (%)	Carbohydrate (%)
AP	72.48±0.67[a]	65.15[b]±0.27	1.92[c]±0.02	14.80[d]±0.02	18.13[e]±0.28
OR	72.90±0.25[a]	65.30[b]±0.27	1.92[c]±0.03	14.81[d]±0.03	17.97[e]±0.35
KR	72.03±1.20[a]	65.13[b]±0.59	1.93[c]±0.02	14.80[d]±0.03	18.13[e]±0.59

Average of six replications expressed on dry weight basis.

Mean values with same superscript do not differ significantly

Table 70 ANOVA of moisture content of body of juveniles of different stocks.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability p
Replication	5	6.20	1.24	3.50	0.09
Population and hybrids	2	2.25	1.13	3.18*	
Residual	10	3.55	0.35		
Total	17	11.11			

*Not Significant (p>0.05)

LSD (0.05) for population means 0.766

Table 71 ANOVA of crude protein content of body of different stocks of juvenile *M.rosenbergii*

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability p
Replication	5	1.59	0.32	2.85	0.65
Population and hybrids	2	0.10	0.05	0.44*	
Residual	10	1.12	0.11		
Total	17	2.81			

*Not Significant (p>0.05)

LSD (0.05) for population means 0.4299

Table 72 ANOVA of crude lipid content of body of different stocks of juvenile *M.rosenbergii*

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability p.
Replication	5	0.0040444	0.0008089	1.48	0.621
Population and hybrids	2	0.0005444	0.0002722	0.50 *	
Residual	10	0.01	0.0005456		
Total	17	0.01			

*Not Significant (p>0.05)

LSD (0.05) for population means 0.03005

Table 73 ANOVA of crude ash content of body of different stocks of juvenile *M.rosenbergii*

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability p.
Replication	5	0.0009778	0.0001956	0.22	0.906
Population and hybrids	2	0.0001778	0.0000889	0.10*	
Residual	10	0.0088889	0.0008889		
Total	17	0.0100444			

*Not Significant (p>0.05)

LSD (0.05) for population means 0.03835

Table 74 ANOVA of crude carbohydrate content of body of different stocks of juvenile *M.rosenbergii*

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability p
Replication	5	1.60	0.32	2.86	0.639
Population and hybrids	2	0.10	0.05	0.47 *	
Residual	10	1.12	0.11		
Total	17	2.82			

*Not Significant (p>0.05)

LSD (0.05) for population means 0.4301

4.3.3 Percentage Survival of *M.rosenbergii* Juveniles (in cage)

The mean survival rates of different populations and hybrids of juvenile *M. rosenbergii* in cage were evaluated and presented in Table 75. Graphical presentation of the same is given in Fig. 24. Maximum survival rate was recorded for AP population (96.6%) and minimum for APKR with 73.35%. Statistical analysis using ANOVA (Table 76) showed significant variation ($p<0.001$) among the populations. LSD test revealed significant difference ($p<0.05$) among all populations except between KR and KROR.

Table 75 Percentage survival rate and percentage weight gain of different populations and hybrids of *M. rosenbergii* juveniles in cages

populations	Percentage survival in Cage	Percentage weight gain in cage (g)
AP	96.60^{e}± 0.64	441.22^{c} ±27.64
OR	91.04^{c}±0.89	582.92^{b} ±8.80
KR	89.60^{b}±0.67	680.08^{a}±25.41
APOR	93.27^{d}±0.54	454.00^{c} ±10.61
APKR	73.35^{a}±1.29	293.57^{d} ±28.18
KROR	89.79^{b}±0.61	718.13^{a} ±35.93
Mean±SD	88.94±8.08	528.32±151.18

Values are mean±SD of six replications

Mean values with same superscript do not differ significantly.

Table 76 ANOVA of percentage survival rate of different populations and hybrids of *M. rosenbergii* juveniles in cages

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability p
Replication	7	7.1224	1.0175	1.73	<.001
Population and hybrids	5	2607.8785	521.5757	884.75*	
Residual	35	20.6331	0.5895		
Total	47	2635.6339			

*Significant ($p<0.001$);

Data subjected to angular transformation.

LSD (0.05): 0.779

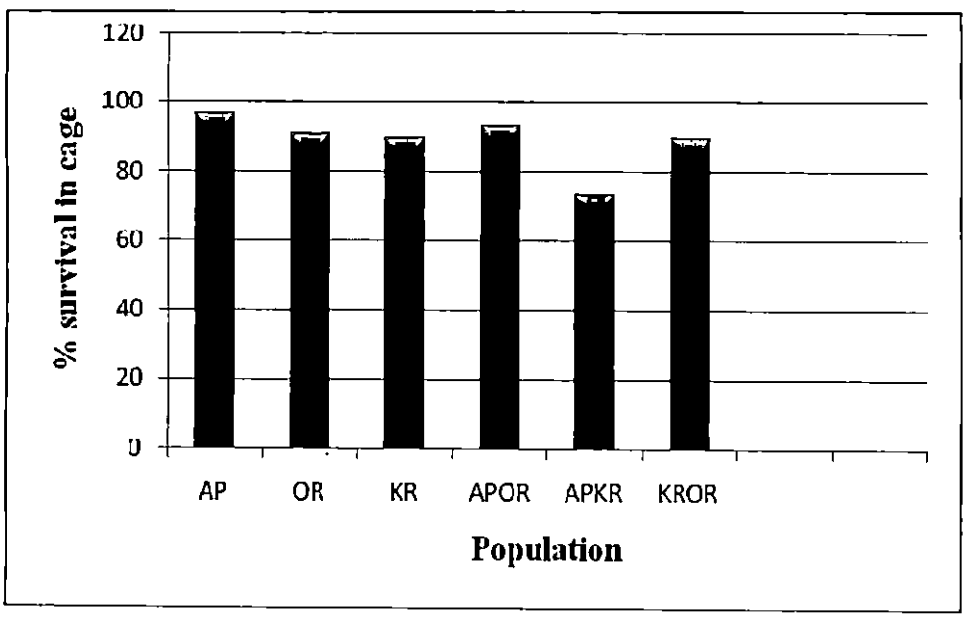

Fig 24 Survival rate of different populations of *M. rosenbergii* juveniles in cages

4.3.4 Percentage Weight Gain in Cages.

The mean weight gain of different populations and hybrids of juvenile *M. rosenbergii* in cage culture were estimated and the results are given in Table 75. Weight gain in cages is diagrammatically shown in Fig. 25. Maximum weight gain was observed for KROR (718. 13 g) followed by KR (680.08 g), OR (582.92 g), APOR (454.0 g), AP (441.22 g) and APKR (293.57 g). Analysis of variance (Table 77) followed by LSD test showed significant variation ($p<0.001$) between populations and hybrids. LSD test revealed that KROR was on par with its parent population KR similarly APOR was on par with its parent population AP. But all others differed significantly in weight gain.

Table 77 ANOVA of weight gain of different populations and hybrids in cage culture

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability p
Populations and hybrids	781569.53	5	156313.91	254.58*	.000
Within Groups	18420.57	30	614.02		
Total	799990.10	35			

*Significant ($p<0.001$);

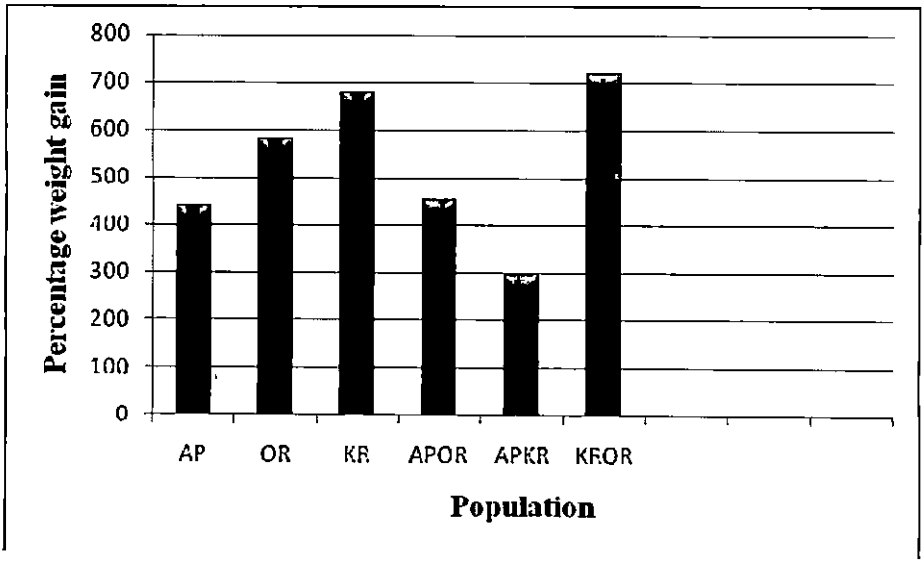

Fig 25 Percentage weight gain of *M.rosenbergii* juveniles (in cage)

4.3.5 Percentage Morphotypes of *M. rosenbergii*

The data pertaining to percentage blue clawed (BC) morphotypes of *M. rosenbergii* of different populations are given in Table 78. Graphical presentation of percentage blue clawed male populations are shown in Fig. 26. Though there was a slight variation in % BC prawns among populations and hybrids. Analysis of Variance of data (Table 79) showed no significant difference between the populations and hybrids

Percentage orange clawed (OC) male were evaluated in the populations and the data are given in Table 78, showing percentage orange a clawed male population is given in Fig. 27. Maximum OC was found in AP followed by KROR, while lowest number was found in OR. Analysis of variance (Table 80) and LSD test showed that AP, having maximum OC is significantly higher than KROR ($p<.05$). But APOR was on par with APKR and KR. Similarly, OR was on par with KR.

Percentage of small males (SM) obtained for different populations are summarised in Table 78. The same is shown in Fig. 28. From the table it is clear that the highest percentage of small males was obtained in OR followed by KR, APKR, APOR, KROR and AP. Analysis of Variance (Table 81) and LSD test revealed significant variation between OR and KR, but KR was on par with APKR and APOR. The population showing lowest number of SM was AP, which was significantly different from other populations. At the same time, there was no significant difference between KROR and APOR.

Percentage females (FM) in each population were recorded and given in Table 78. Diagram showing of percentage female populations is given in Fig. 29. It is found that the highest percentage of female prawns was in KROR and lowest in OR. Analysis of variance (Table 82) of the data showed no significant variation ($p>0.05$) between populations.

Table 78 Percentage morphotypes in three stocks and hybrids of *M. rosenbergii* from pond

Populations	BC male (%)	OC male (%)	SM (%)	FM (%)
AP	$43.65^{a}\pm2.44$	$20.39^{e}\pm1.82$	$9.38^{f}\pm1.17$	$26.58^{i}\pm2.92$
OR	$42.08^{a}\pm1.87$	$15.29^{b}\pm1.31$	$16.91^{i}\pm2.15$	$25.73^{j}\pm2.69$
KR	$43.27^{a}\pm2.28$	$16.34^{bc}\pm0.90$	$13.84^{h}\pm1.26$	$26.55^{j}\pm2.27$
APOR	$43.89^{a}\pm1.01$	$16.90^{cd}\pm0.90$	$12.47^{gh}\pm1.33$	$26.70^{j}\pm1.10$
APKR	$43.55^{a}\pm1.05$	$16.87^{cd}\pm1.02$	$13.22^{h}\pm1.20$	$26.35^{j}\pm2.01$
KROR	$43.29^{a}\pm1.84$	$18.02^{d}\pm0.65$	$11.56^{g}\pm1.23$	$27.12^{j}\pm1.82$

Mean±SD of eight replications;
Mean values with same or common superscript do not differ significantly.

Table 79 ANOVA of percentage blue clawed male morphotype of populations and hybrids.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability (p).
Replication	7	42.68	6.10	2.17	0.352
Population and hybrids	5	16.21	3.24	1.15*	
Residual	35	98.55	2.82		
Total	47	157.42			

*Not Significant (p>0.05)
LSD(0.05): 1.703

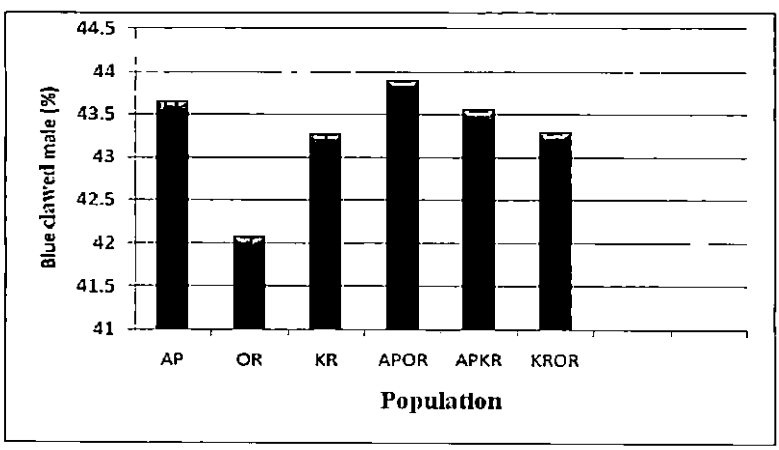

Fig 26 Percentage BC male morphotype of populations and hybrids of *M. rosenbergii* from pond

Table 80 ANOVA of percentage orange clawed male morphotype of populations and hybrids

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability p.
Replication	7	0.78	5.46	0.53	<.001
Population and hybrids	5	123.29	24.66	16.81*	
Residual	35	51.34	1.47		
Total	47	180.10			

*Significant($p<0.01$)

LSD (0.05): 1.229

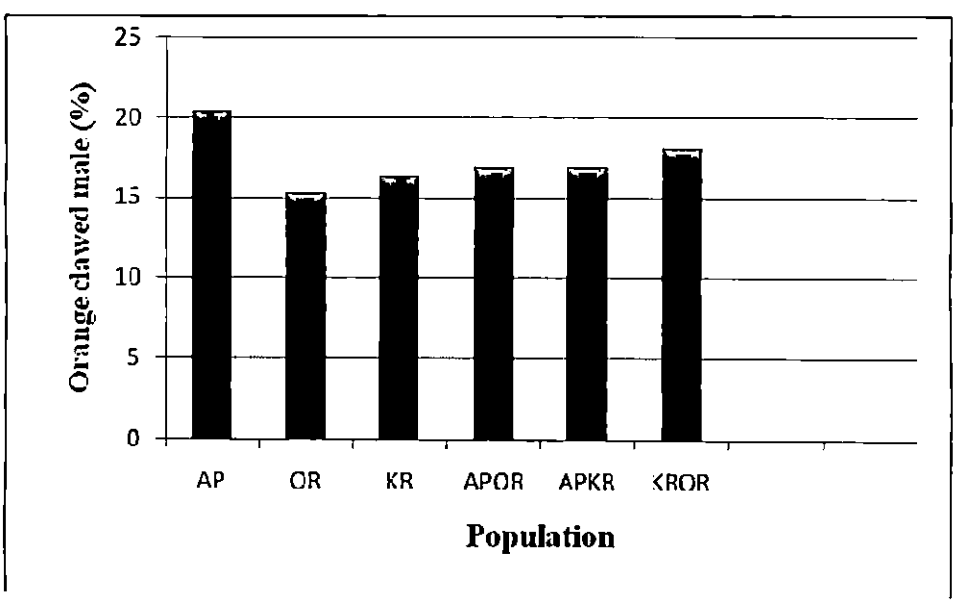

Fig 27 Percentage orange clawed male morphotype of populations and hybrids of *M. rosenbergii*

Table 81 ANOVA of percentage small male morphotype in population and hybrids

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	probability. p
Replication	7	11.10	1.71	0.81	<.001
Population and hybrids	5	251.47	50.29	23.72	
Residual	35	74.20	2.12		
Total	47	337.67			

*Significant ($p<0.001$)

LSD (0.05): 1.478

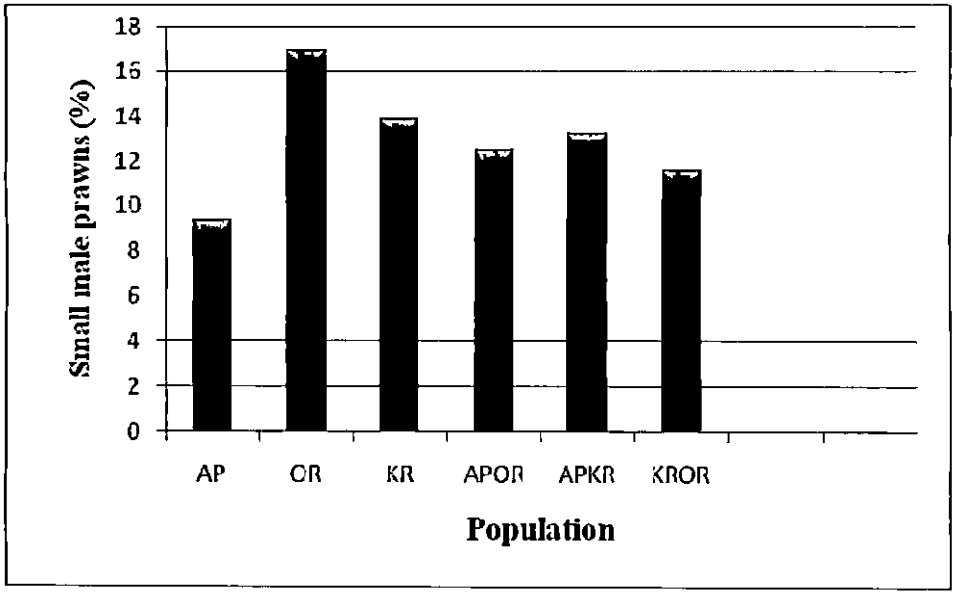

Fig 28 Percentage small male morphotype of populations and hybrids of *M. rosenbergii* .

Table 82 ANOVA of percentage female morphotype of populations and hybrids

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability p.
Replication	7	45.44	6.49	1.41	
Population	5	8.48	1.70	0.37 *	
Residual	35	160.76	4.59		0.866
Total	47	214.69			

*Not Significant (p>0.05)

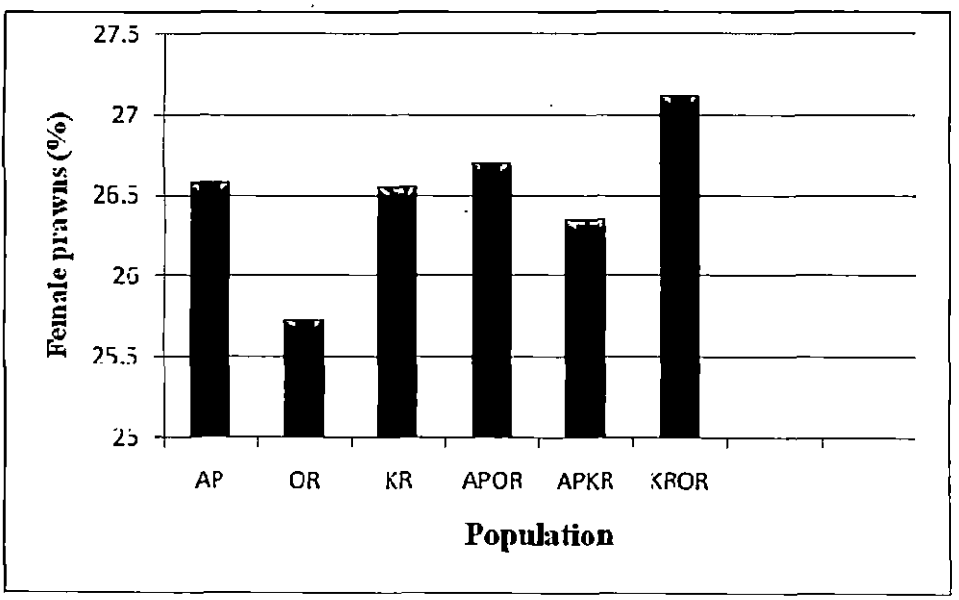

Fig 29 Percentage female morphotype of populations and hybrids of *M.rosenbergii*

4.3.6 Head –Tail Weight Ratio

The data pertaining to head-tail weight ratio of different populations were estimated and summarised in Table 83 and its graph is shown in Fig. 30. From the table it is observed that the lowest head -tail weight ratio was for KROR (1.23) followed by OR and APOR while highest ratio was observed for AP (2.415) followed by KR and APKR. Analysis of variance (Table 84) of the

ratios revealed that there is significant variation between the populations ($p<0.001$). Population KROR having lowest ratio was on par with OR, APOR, APKR and KR. Similarly KR, APKR, showed no significant difference.

Table 83 Head- tail weight ratio of different populations and hybrids of *M. rosenbergii* .

Populations	Head-tail weight ratio (Mean±SD)
AP	2.42^{b} ±0.132
OR	1.25^{a}±0.151
KR	1.38^{ab}±0.191
APOR	1.30^{a}±0.024
APKR	1.36^{ab}±0.031
KROR	1.23^{a} ±0.056

Mean±SD of eight replications
Mean values with same or common superscript do not differ significantly.

Table 84 ANOVA of head- tail weight ratio of different populations of *M. rosenbergii*

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability p
Replication	7	0.16	0.02	1.98	<.001
Population and hybrids	5	8.38	1.68	145.87	
Residual	35	0.40	0.01		
Total	47	8.94			

*Significant ($p<0.001$)

LSD (0.05): 0.1088

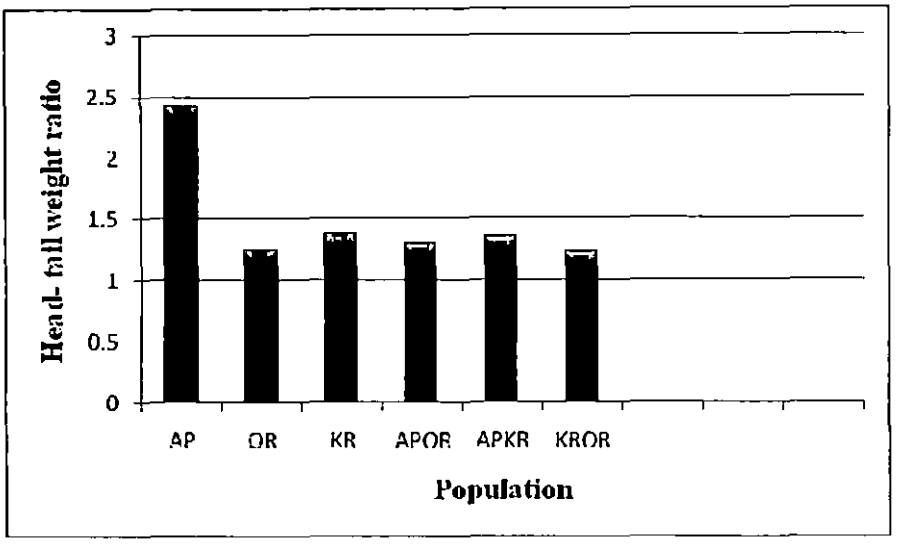

Fig 30 Head- tail weight ratio of different populations and hybrids of *M. rosenbergii* adults

4.4 HERITABILITY OF WEIGHT

Mean weight of parents, hybrids and heritability of weight of hybrids (APOR, KROR and APKR) are given in Tables 85, 86 and 87. From the tables it is found that the highest heritability value was for KROR (0.75) followed by APOR (0.16) and APKR (0).

Table 85 Heritability of weight (hybrid APOR)

Weight of parents(g)		Average weight (g)		Heritability
AP	OR	Parents	Hybrid APOR	
95.50	100.00	97.75	45.33	
101.20	105.00	103.10	47.52	
101.23	95.00	98.12	48.42	
98.60	98.00	98.30	46.56	
98.80	100.00	99.40	46.98	**0.161**
102.40	102.6	102.50	48.20	
100.80	103.00	101.90	48.59	
96.97	95.80	96.39	47.99	

Table 86 Heritability of weight (hybrid KROR)

Weight of parents(g)		Average weight (g)		Heritability
KR	OR	Parents	Hybrid KROR	
98.50	104.50	101.50	40.661	0.75
99.60	100.80	100.20	42.57	
97.680	103.00	100.34	38.56	
100.30	101.00	100.65	45.73	
100.20	102.00	101.10	44.53	
99.60	95.00	97.30	39.84	
100.60	98.00	99.30	40.65	
99.90	102.50	101.20	42.45	

Table 87 Heritability of weight (hybrid APKR)

Weight of parents (g)		Average weight (g)		Heritability
AP	KR	Parents	Hybrid APKR	
100.40	105.00	102.70	64.27	0
99.50	102.70	101.10	60.59	
103.30	103.40	103.35	62.89	
99.40	100.00	99.70	63.98	
101.10	100.80	100.95	64.46	
102.20	99.60	100.90	64.66	
101.60	101.50	101.55	62.73	

Discussion

5. DISCUSSION

5.1 GENETIC CHARACTERIZATION

5.1.1 RAPD Markers

Random amplified polymorphic DNA (RAPD) is a multi-locus dominant marker, which provides multiple markers without any prior knowledge of the DNA sequences. Short primers of random eight to ten base pairs at lower annealing temperature are used to amplify the DNA in Random polymorphic DNA (RAPD). The results of the attempt to describe the RAPD patterns are discussed.

5.1.2 Yield and Quality of DNA

The yield of DNA obtained from the tissues of prawns from different regions was compared. The average yield of DNA from Andhra samples were comparable to Orissa followed by those from Kerala.

The purity of DNA was estimated by the ratio of absorbance reading between 260 and 280 nm (OD260/OD280) using UV spectrophotometer. OD ratio was taken as an indicator of quality of DNA. The ratio obtained for Andhra Pradesh, Orissa and Kerala were 1.821±0.063, 1.8 ±0.046 and 1.81 ± 0.043 respectively.

5.1.3 Primers Used

In this study the five primers of 10bp length were used for RAPD-PCR. These oligonucleotides serve as both the forward and reverse primers. The variable lengths of the amplified fragments of DNA are inherited as classical Mendelian traits (Williams *et al.*, 1990) and thus it can be used for genetic analysis (Horn *et al.*, 1996). But the amplification products depend on the length and sequence of primers and reaction conditions.

See *et al.*, (2008) used 5 primers for the analysis of polymorphism among 11 populations of *M. rosenbergii* populations.

5.1.3.1 Primer OPA1

The primer OPA1 produced twelve bands for Andhra samples, eleven bands for Orissa population and eleven for Kerala. Andhra populations produced five rare bands having frequency less than 0.25, while Orissa samples exhibited six and Kerala four rare bands. This primer is highly polymorphic. Hence the primer is highly effective for studies on genetic polymorphism in *M.rosenbergii* samples. Three, four and four numbers of bands having frequency more than 0.5 were produced by the primer in Andhra, Orissa and Kerala stocks respectively, which are having limited use in genetic polymorphism studies. Moreover there were total six monomorphic bands in all the three populations having frequency of 1, with no use in the studies of genetic polymorphism. See *et al.* (2008) also used OPA1 primer for the genetic analysis of different populations of *M. rosenbergii*.

5.1.3.2. Primer OPA3

Primer OPA3 had nine bands each for Andhra and Orissa samples and ten bands for Kerala sample. One band for Andhra and two bands for Orissa were commonly produced by the primer. But OPA3 did not produce any common band for Kerala population. This indicates that this primer is monomorphic to Orissa and Andhra Pradesh. Presence of four bands having frequency less than 0.25 in Orissa sample indicate the usefulness of this primer for genetic polymorphism studies. But the numbers of rare bands were less for Andhra and Kerala. But in Kerala sample, the number of bands having frequencies 0.25-0.50 and 0.50-1 were five and four respectively. This makes the primer effective in genetic polymorphism analysis. One monomorphic band for Andhra and two monomorphic bands for Orissa with frequency 1 were produced, which have no use in the analysis of polymorphism. But Kerala samples did not produce any bands having frequency 1.

5.1.3.3. Primer OPA7

The number of bands produced by the primer OPA7 was nine each for Andhra and Orissa and eleven for Kerala population. No monomorphic bands were present in any of the samples of Andhra Pradesh and Kerala, but OPA7k was present as common band in all samples of Orissa. This primer OPA7 was highly polymorphic in Kerala with five rare bands of frequency less than 0.25 and two bands of frequency between 0.25 to 0.5. It is also fairly polymorphic in Orissa and Andhra Pradesh with two and three bands of frequency less than 0.25. This reveals the utility of this primer in the studies of population genetics. Moreover Kerala and Andhra Pradesh samples did not produce any bands having frequency 1.

5.1.3.4. Primer OPA9

Total number of band produced by the primer OPA9 is comparatively less in Andhra (four bands) and Orissa (seven bands); but it produced nine bands in Kerala samples. The number of rare bands of Orissa (five bands) was very high followed by Andhra Pradesh (three bands) and Kerala (one band). In Kerala samples, bands having frequency less than 0.25 was low, but number of bands having frequency between 0.25-0.5 were high (five bands.) and there was no band having frequency 1. These results indicate that the primer is suitable for genetic polymorphism studies.

5.1.3.5. Primer OPA10

The primer OPA10 produced highest number of band in Andhra samples (fourteen bands) followed by Kerala (thirteen bands) and Orissa (ten bands). Kerala and Andhra populations produced four bands with frequencies less than 0.25, while Orissa produced two bands. This primer also produced three bands having frequency between 0.25-0.50 in Kerala samples. But all the three populations showed large number of monomorphic bands, which were of

nouse in the studies of genetic polymorphism. Hence the use of this primer is limited in population genetic studies of different populations of *M. rosenbergii*.

5.1.4 RAPD Analysis

From RAPD studies it is clear that all the three populations (AP, OR and KR) were genetically different. From the analysis of six populations - analysis it was found that maximum within population gene diversity was in KAK population and AP. Shannon Information index, values of different populations revealed genetic variation within the population and was found that maximum for KAK followed by AP and KCH.

The data of pair wise comparison of similarity index and genetic distance, it was found that the higher genetic distance was observed between AP and KR populations except KAK. It was observed that KAK showed less genetic distance and more similarity with AP stock. This could be explained on the basis of the reports of ranching of giant freshwater prawn seed in the rivers of Kerala, the State Fisheries Department has been undertaking since 1998. A good number of prawn postlarvae brought from hatcheries located in Andhra Pradesh might have gained entry to some of the rivers in Kerala by way of ranching, perhaps causing mixing with the native gene pool.

The second dendrogram divide the population into three major groups. The first cluster belongs to Andhra Pradesh (AP); Orissa formed the second group and Kerala population formed the third group. From the data it is found that maximum within population gene diversity was for KR than that of OR and AP. Genetic diversity in wild *M. rosenbergii* stocks is high and structured spatially among major river drainages (De Bruyn *et al.*, 2004a, b, 2005; De Bruyn and Mather, 2007). In this analysis it is found that the genetic distance between AP and KR was lower than that of the same between AP and OR. This may be due to KAK population, which showed low genetic distance value.

Number of polymorphic loci and percentage polymorphism were also found maximum in KR population. This indicates that KR population shows high genetic diversity and this variation in wild stocks could be exploited in breeding programs to develop improved lines. Crossing highly divergent populations might result in heterosis, and this could be used as a convenient and inexpensive tool for selecting strains of superior performance, and could often be used as an economic alternative to the cumbersome process of selective breeding. Fjalestad (2005) stated that the magnitude of heterosis depends on the level of genetic differentiation among parental populations. RAPD analysis of *M.rosenbergii* was done by See *et al.* (2008) in eleven populations of prawns. From the analysis they found that there were three clustering among all the populations.

5.1.5 Microsatellite Analysis

The microsatellite markers are highly polymorphic, relatively abundant and are less sensitive to PCR assay, hence it is more reliable over other markers. Microsatellite markers was found to be very useful for verifying pedigrees in prawn lines (Moore *et al.*, 1999) and is considered as a valuable tool in the investigation of genetic diversity and pedigree tracing of hatchery populations (Perez-Enriquez *et al.*, 1999).

5.1.5.1 Primer Mbr 4

In this study Mbr 4 locus produced 20 alleles in Andhra stocks having size ranging from 216 to 250 base pairs, 19 alleles in Orissa stock (206-240 bp) and 21 alleles in Kerala stocks (210-300 bp).

5.1.5.2 Primer Mbr 10

The allelic size of Mbr 10 locus in this study has been found to lie in between150 to 252 base pairs in Andhra population with 26 alleles, while in Orissa population this primer produced 29 alleles of size ranging from 150 to 250 base pairs and in Kerala 51 alleles of size 150-252 base pairs.

5.2 COMPARATIVE LARVAL PERFORMANCE

5.2.1 Egg diameter

In this experiment egg diameter of three populations and their six hybrids was estimated. Maximum egg diameter was observed in hybrid KROR. But this value did not show any significant variation with its parent populations (KR and OR) and it's reciprocal cross ORKR. This cross showed slightly higher diameter than its parent populations while it's reciprocal cross ORKR exhibited intermediate value of egg diameter between parent values. Similarly other hybrids APOR, ORAP, KRAP showed lower egg diameter values, which differed significantly from parental populations and other hybrids. This variation in egg diameters of base population may not be due to environmental factors or water quality, because all the parents were reared and bred under identical conditions. Moreover, the egg diameter of hybrids exhibited a correlation with parent egg size. This indicates that this trait is controlled by quantitative genetic system. Hence egg size seemed to be controlled by genetic variation.

In the case of all hybrids, variation in egg diameter was found among reciprocal crosses. A general consensus is that in any aquatic species, variation between the relative performances of different reciprocal strain crosses or species can largely be attributed to maternal effects (Lutz, 2001). Relative egg size and egg quality, and relative maternal skill in caring for eggs or fry are main traits, which are affected by maternal factors. This finding is in agreement with the present study. OR and KR populations showed better performance in egg diameter, while AP showed smaller egg diameter. Similar effects were observed in hybrids also. Hybrids where the KR and OR contributing the maternal factor, which showed large egg diameter compared to its reciprocal crosses. It is often difficult however, to determine the specific factors that influence maternal effects in most cases.

Mashiko (1992) conducted similar studies in two populations of *M nipponense* about genetic basis of egg and clutch sizes and reported similar results. Egg size of prawns of different populations differed remarkably and hybrids exhibited intermediate egg sizes of parents. Reproductive traits of egg size and clutch size have been conducted in caridian shrimps. Yam and Dudgeon (2005) conducted work on inter and intra specific variation in life history and growth of *Caridina spp.* and they reported variation in brood size but not in egg size of different population.

Clutch and egg sizes are fundamental life history traits, which directly determine population or individual fitness, especially in aquatic invertebrates where parents do not nourish the young ones (Mashiko, 1992). Intraspecific variations in these reproductive traits have been noted in many species of decapods crustaceans (Efford, 1969; Nishino, 1980) and lower crustaceans (Lonsdale and Levinton, 1985; Belk *et al.*, 1990).

5.2.2 Larval Hatch Fecundity

Successful hatchery management requires knowledge of the number of brood stock required to produce a defined number of postlarvae. The purpose of this study is to compare the larval hatch fecundity of *M. rosenbergii* from different regions of India. In the present experiment, the highest average number of viable larvae per female was recorded for Kerala stock and least for KRAP hybrid. There was no significant difference between the hatch fecundities of KR, AP and OR prawns. But the hatch fecundities of hybrids were low except for KROR. In this study the weights of brooders used for the production of larvae were more or less same in the case of all the hybrids and base populations. Among the hybrids the highest hatch fecundity was reported for KROR, hence it can be considered as the best among various hybrids. Fecundity indices can be used for the quantification of brood stock requirement. Brooders with high hatch fecundities will help to reduce the number of brooders in hatchery operations.

New and Singholka (1985) and D' Abramo *et al.* (1995) reported that each gram of berried female of *M.rosenbergii* gave 1000 numbers of larvae. These observations are in agreement with the hatch fecundity of wild stocks and the hybrid (KROR) estimated in the present study. But Malecha (1983) and Brooks (2001) have reported a lower larval hatch fecundity of *M.rosenbergii* as 400 and 405. Dinesh and Nair (2003) found higher larval output for wild caught berried prawns than that of pond reared ones. They concluded that this may be due to the better conditions prevailing in natural water bodies compared to the stressed and overcrowded condition in the pond. But in this study, wild stocks domesticated for one generation were reared and bred under similar pond condition. Hence the high hatch fecundity may be due to genetic divergence of the stocks. The results of the present study indicate that the hatch fecundity of hybrid KROR is as good as the wild stocks of prawns.

5.2.3 Larval Rearing Period

The data of the present study clearly indicate that the mean time taken for first PL metamorphosis of Andhra stock (AP) and its hybrid with Kerala (APKR) have shorter and Orissa have longer larval rearing period. But other hybrids of Andhra stocks showed long larval rearing periods. Larval rearing phase is the most important and critical stage for a commercial hatchery, which can have direct correlation with survival and production cost (Woods *et al.*, 1998; Dasgupta and Tidwell, 2003). Hence shorter larval rearing period of freshwater prawn helps to reduce the production cost.

Reduction in mean time taken for first PL metamorphosis of Andhra and Andhra - Kerala hybrid (APKR) might be due to its small and more or less uniform size. Uniform size of larvae must have created a better feeding atmosphere, which helps in early metamorphosis of larvae to PL. Bart and Yen (2003) found similar results in the larval development of Taiwanese and Vietnamese stocks. Vietnamese stocks showed high differential growth and

slow development as in the case of Orissa (OR) stock. The first post larvae appeared in a shorter period when brooders were maintained in 0 ppt (22.66 days) and 5 ppt (24.66 days) than brooders kept in higher salinity (Sundarapandian *et al.*, 2009).

5.2.4 Larval Survival

From the present study, it has been found that maximum survival rate was for hybrid APOR followed by AP and minimum for KRAP. Survival rate of Kerala (KR) and Orissa (OR) was low compared to Andhra stock (AP). One of the constraints of culture of freshwater prawn has been variable larval survival, which is assumed to be directly linked with good water quality, quality and quantity of food and the ability of prawns to acquire food (Armstrong *et al.*, 1976; Aquacop, 1983; New, 1990). In this study water quality parameters, quality and quantity of feed were uniform in all the treatments. Hence here the survival difference could be due to the relative difference in the ability of larvae to collect feed. This may be due to highly differential metamorphosis among KR and OR stocks compared to AP. The earlier metamorphosis among OR and KR stocks could have socially suppressed the small ones in the larval population not making available sufficient feed to them, which could ultimately result in reduced survival rate in the culture. Moreover, differential growth will cause high rate of cannibalism and that may be another cause of low larval survival rate. Similar result was reported in Vietnamese larvae, which showed more differential growth by Bart and Yen (2003). They also reported short span of development of Taiwanese stock compared to Vietnamese stock, which shows slow or lower uniform rate of development. The survival rate of *M.rosenbergii* larvae was significantly influenced by water salinity (Soundarapandian *et al.*, 2009).

5.2.5 Larval Length

Progressive increase in the size of larvae of different populations and hybrids were evaluated. From the present study it is found that the parent populations KR and OR have the maximum larval length compared to AP. Similar effects are seen in hybrids also. Hybrids of AP showed lower larval length compared to Kerala and Orissa hybrids. The results show that variation in genetic makeup among parent populations of prawns influenced the growth rate of larvae. Hybrid KROR exhibited maximum growth compared to its reciprocal cross ORKR. This is the evidence of effect of heterosis for some cross combinations. Growth is a quantitative trait, which is controlled by more than one gene. Hence transfer of that trait to next generation is not similar in all crosses may be the reason for less growth of ORKR compared to KROR. Similar results in larval development of US prawns were reported by Malecha (1983), Sandifer and Smith (1985).

Differential performance of reciprocal crosses has been quite common in aquatic species, including finfish (Gjerde, 1988; Bentsen *et al.*, 1998; Gjerde *et al.*, 2002; Bryden *et al.*, 2004), crustaceans (Bosworth *et al.*, 1994; Yi *et al.*, 2006) and molluscs (Hedgecock and Davis, 2007; Zhang *et al.*, 2007). Hedgecock and Davis (2007) reported that reciprocal effects can be divided into maternal and non-maternal components, and a significant non-maternal contribution to reciprocal variance would indicate strong interactions between extra-nuclear and nuclear factors such as interactions between mitochondrial and nuclear genes.

5.2.6 Stress Tests

Stress tolerance studies demonstrate the relative differences in the ability of different populations to withstand unfavourable stressed conditions.

5.2.6.1 Temperature tolerance test

Different populations and hybrids were subjected to different temperatures under identical conditions and from the results of temperature stress test. This study found that KR and KROR showed higher LT_{50} values than all other populations and hybrids. In the fifth stage of larvae, maximum larval mortality number was recorded for AP and minimum for Kerala followed by OR at all temperatures to which the larvae were exposed. From the analysis it was also found that KR stock and its hybrids KROR and OR were more tolerant to thermal stress in all temperatures and AP stock and its all hybrids were more susceptible to temperature stress. Similarly it was also found that a direct relationship between stages (body size) and thermal tolerance of larvae. The LT_{50} value of KROR and KR in V and X stages were (37.36 ^{0}C, 37.52 ^{0}C) and (37.36 ^{0}C, 37.45 ^{0}C), respectively. Rahman *et al.* (2004) conducted stress resistance studies in the larvae of freshwater prawn and they reported a LT_{50} of 38.5 ^{0}C. Temperature tolerance study helps to compare the performance of different populations and their hybrids, which provides an idea about its performance during transportation and in field condition.

Temperature is a crucial factor which affects growth, molting rate and feed intake. The optimal temperature for growth of *M rosenbergii* is 29- 31^{0}C (Chen and Chen, 2003). It is reported that small changes in water temperature can have considerable effect on freshwater fishes (Morgan *et al.*, 2001). A temperature increase beyond the optimal range for any species can influence the capacity to function properly (Crawshaw, 1977). The thermal tolerance of an organism is influenced by many biotic and abiotic factors, acclimation temperature and thermal history are among the most important (Beitinger *et al.*, 2000; Chung, 2001). It is not divide the crustacean species into thermal guilds as in the case of fishes. Thermal preference contributes to fitness in different ways in different species.

5.2.6.2 Salinity tolerance test

Results of salinity tolerance study revealed that larvae of all the hybrids except APKR in V stage and APKR and KRAP in X stage showed better salinity tolerance than that of parent populations. LC_{50} values of KROR in V and X stage were 36.04 and 41.31. Fujimura, (1974) and Goodwin and Hanson (1975) reported a salinity tolerance of *M.rosenbergii* larvae upto 21 ppt, New (1995) 25 ppt and de Bruyn *et al.* (2004) 15 ppt. Bart and Yen (2003) conducted salinity stress test in two stocks of *M. rosenbergii* larvae and they reported no significant variation in salinity tolerance of larvae. During early development, *Macrobrachium idae* larvae preferred salinities from 5 to 20 ppt. In these salinities all larvae moulted after 24 h; their median tolerance (Subramanian *et al.*, 1980).

Salinity tolerance is one of the most important fitness-related traits in fish. Shikano *et al.* (1997). In many studies salinity tolerance was used to quantify the level of inbreeding depression and heterosis due to its strong relation with inbreeding coefficient. Usually salinity tolerance decreases with the intensity of in breeding. (Shikano and Taniguchi, 2003). Therefore, they proposed that the trait of salinity tolerance is one of the most useful indicators to study the amount of heterosis in crosses and inbreeding depression in populations. In this study the higher salinity tolerance of larvae of hybrids than parent stock is due to hybrid vigour. But similar results were not found in APKR and KRAP. Among parent populations KR exhibited higher tolerance than others followed by OR and AP. This difference may be due to genetic variation and that trait was found to transfer from parent to offspring and hybrids of KR and OR also showed maximum tolerance in salinity.

In addition to this a stage wise variation in salinity tolerance was also clear in this study. Larvae of all the populations and hybrids showed better tolerance in X stage than V. Salinity is the ecological key factor in the life cycle of *M. rosenbergii,* which inhabits freshwater but the larval and post larval

phases are spent in brackish water. Thus, the degree of tolerance towards environmental factors may differ according to phase (Cheng and Chen, 1998a).

5.2.6.3 Formaldehyde tolerance test

Formaldehyde stress tests in larvae of *M.rosenbergii* were also showed same patterns of tolerance. Highest tolerance was found in KROR (286.65 mg/l) in V stage and 361.88 mg/l in X stage. Variation in tolerance level was observed in parent population also. It can be taken as a fitness trait. Hence from the result it is clear that KR population was highly tolerant followed by OR. Bart and Yen (2003) evaluated the formalin tolerance of two stocks of *M. rosenbergii* larvae and they found no significant variation in formalin tolerance of larvae. Samocha *et al.* (1998) used formalin stress test as an index for larval health in *Litopenaeus vannamei.*

5.2.6.4 Ammonia tolerance test

Ammonia toxicity study is conducted to determine the effect of ammonia on different populations and hybrids. More over it plays an important role in crustacean rearing, since ammonia concentrations in culture systems may often exceeds in levels beyond limit (Spotte, 1970). In this study V and X stage of *M.rosenbergii* larvae were continuously subjected to different concentrations of Total ammonia for 3 h. and corresponding mortality rates were recorded. From the results it was found that maximum LC_{50} value in V stage was for KR (140.25 mg/l) followed by KROR (139.21 mg/l). But in X stage KROR (132.66 mg/l) showed slightly higher value than KR (132.29 mg/l). Similar studies were conducted by Wickins (1976) and found that 101 mg ammonia/litre at pH 7.0 gave an LC_{50} of 24 h for adult *Macrobrachium.* Armstrong *et al.* (1978) reported the 24 h LC_{50} values 200, 115 and 37 mg ammonia/litre at pH 6.83, 7.60 and 8.34, respectively. But Rahman *et al.* (2004) reported the same survival level for the larvae of *M.rosenbergii* before and after heat shock when challenged with ammonia toxicity.

Cavalli *et a.l* (2000) conducted short term ammonia toxicity test for assessing larval quality of freshwater prawn and found that prawns fed with nutritious diet showed better tolerance. But in this study the prawns of different populations and hybrids were reared under identical conditions and fed with same feed. The variations in tolerance level of larvae in this study were not due to nutritional factors, but clearly due to variation in genetic structure. In this study it is also found that ORKR showed more susceptibility towards ammonia toxicity than KROR. From this finding it is clear that cross combination plays a crucial role in the transfer of hybrid vigour in these hybrids, for this particular trait.

Another factor found in this study was the larvae of V stage of all populations and hybrids showed remarkably higher tolerance to ammonia than X stage. This is in agreement with the results of Mallasen and Valenti (2005). They conducted ammonia toxicity studies in *M.rosenbergii* larvae and found that they could tolerate high levels of total ammonia in early larval stages than late zoeal stages.

5.3 COMPARISON OF ECONOMICALLY IMPORTANT TRAITS

5.3.1 Disease Resistance

5.3.1.1 Viral challenge study

Tolerance to viral diseases of different populations was studied. From the data is found that the minimum mortality was recorded in KR and its hybrid KROR followed by OR. At the same time AP was less tolerant to white tail virus. Similarly Andhra crosses also showed more susceptibility to viral diseases. These populations and hybrids were subjected to challenge studies under similar conditions. Hence the variation in viral tolerance may be due to genetic reason. KR and OR populations and its hybrid KROR are having better disease resistance than AP and its crosses. There are six treatments in this study. In first two treatments the prawns were subjected to low concentrations

of virus and here no mortality was observed. But when concentrations of virus increased in subsequent treatments the mortality rate also increased. Similarly in each treatment, mortality rate of populations follows the same pattern as in the case of population studies. That means the effect of different treatments on mortality of population is minimum for KR and KROR.

5.3.1.2 Bacterial challenge study

M. rosenbergii juveniles were subjected to bacterial challenge study with different concentrations of *Enterococcus aerogenes* bacterial inoculum. In this study also the pattern of mortality of different populations, treatment effect and population treatment effects are similar in the case of viral study. This result showed that the populations KR, KROR and OR were bacterial tolerant than that of AP and other hybrids. Sung *et al.* (2000) conducted challenge study in *M. rosenbergii* for evaluating the virulence of two strains of *Aeromonas spp.*and they reported LD-50 value 2×10^3 cells/g for *A.veronii* and 51.2×10^3 cells/g for *A. caviae.*

Here also KROR and KR showed higher values of PPO (Prophenoloxidase). In OR, KR and KROR 3.68, 3.58 and 3.65 times increase in PPO activity from control after bacterial inoculation, while in other prawns the magnitude of increment was comparatively low. Similar finding were reported by Sung *et al.* (2000) in *M.rosenbergii.* In all populations, treated prawns showed a significant increase of PPO activity. The terminal enzyme in the prophenoloxidase (proPO) activation system is activated by many substances including several microbial polysaccharides (Smith *et al.*, 1984), which is detectable in the granular cells (GC) and semi granular cells (SGC) of the giant freshwater prawn (Sung *et al.*, 1998). Phenoloxidase stimulates cellular defence mechanism and plays an important role in melanisation. proPO activation of the system supports the host-defence functions such as phagocytosis (Sung and Sun, 1999), by granular cells and semi granular cells (Sung *et al.*, 1998).

5.3.1.3 Prophenoloxidase activity (PPO)

Many decapods have been shown to rapidly remove large number of injected bacteria from their haemolymph (Factor and Beekman, 1990). Better viral and bacterial tolerance of these populations and hybrids can be justified by the results of pro phenoloxidase activity and haemocyte count of challenged prawns. This increased susceptibility of AP and other hybrids is considered to be related to the decrease in phenoloxidase activity, of prawns infected by *Enterococcus spp.* Environmental factors like temperature, stress etc. affect the number of THC (Total haemocyte count), but here all the prawns were maintained in identical conditions. Hence the variations in the count, PPO and disease resistance may not be due to extrinsic factors. These results of better disease resistance of KR, OR and its hybrid KROR can be clearly due to the variation in genetic makeup and it is also seen that the trait is heritable.

5.3.1.4 Total haemocyte count (THC)

Injection of *Enterococcus aerogenes* (1.1×10^2 cells) after 24 h. led to a significant decrease in total haemocyte count in all population and hybrids of *M rosenbergii* compared to control. But in control highest count was recorded in KR and KROR and a decrease of 3.08 and 3.13 fold in the THC of KR and KROR was recorded in treated prawns. A rapid clearance of bacteria injected in to the haemolymph of shrimp *Sicyonia ingentis* (Martin *et al.*, 1993), *P monodon* (Sung *et al.*, 1998) and *M. rosenbergii* (Sung *et al.*, 2000) has also been reported. Haemocytes play a vital role in the defence response of crustaceans through their participation in coagulation, phagocytosis, encapsulation, and cytotoxic mediation (Hose and Martin, 1989; Bache`re *et al.*, 1995). In shrimps, bacterial clearance is associated with a rapid and significant decline in the number of circulating haemocytes (Smith *et al.*,1984; Martin *et al.*, 1993). Haemocytes may migrate from haemolymph to infected tissue to activate phagocytosis, granulocyte, which may breakdown to activate prophenoloxidase system. The above findings are in agreement with the result

of the present study, where there was a fall of haemocyte count in treated prawns compared to control.

5.3.2 Carcass Composition

Carcass proximate analysis of juvenile prawns of different populations was done. The chemical composition of body of prawns of different populations is not significantly different. The data on percentage protein of present study showed slight variation but not significantly different. Maximum protein was reported for OR population. Sagar *et al.* (2009) reported 55.26 ± 0.26 of protein levels in Andhra prawns. In this work crude protein level of Andhra prawn is 65.15 ± 0.27, which is slightly higher than the above result. Lipid content in the whole body of the juvenile prawns were estimated and found that all the populations were not differed significantly. The lipid levels of AP, OR and KR were 1.92%, 1.92% and 1.93% respectively. The moisture content of body of prawns of different populations are 72.48%, 72.90%, 72.03% for AP, OR and KR respectively. Slightly higher moisture content was found in OR but not shown any significant difference among populations. From the present experiment it was found that carbohydrate content of body of different populations was maximum for KR and AP but not significantly different. Shyla *et al.* (2009) reported more or less similar results of body composition of *M. rosenbergii* juveniles in proximate analysis.

5.3.3. Percentage Survival Rate of *M.rosenbergii* Juveniles (in cage)

To reduce the effect of environmental parameters on growth and survival, different populations and hybrids of *M.rosenbergii* juveniles were reared in cages of same size kept in same pond. From the result of cage culture it is found that highest survival among different stocks was for AP followed by OR and KR, respectively. In the case of hybrids maximum survival rate was recorded in APOR followed by KROR and APKR. Survival rate of APOR is higher than that of OR and KR. The variation in survival rate of different parent populations

was due to their differential growth rates. AP showed least differential growth than that of OR and KR. The differential growth among OR and KR stocks may cause variation of intake of feed by large prawns and cause high rate of cannibalism and that may be another cause of low survival rate. In the case of hybrids APOR and APKR showed minimum differential growth. This may be the reason for higher survival rate of APOR, which was more than that of OR and KR. But the survival rate of APKR was still less. Generally APKR hybrids showed slow growth and were weak in nature. In larval stage also APKR showed low survival rate. Moreover, small male population was more in OR, KR and APKR. But the BC population was almost same. Presence of BC causes growth suppression and survival rate of SM

Naik *et al.* (2003) conducted nursery rearing of prawns in cages and they reported that the growth rate and survival rate of cultured organisms were inversely proportional to stocking rate. Similar observations were reported by Sandifer and Smith (1975), Wills and Berringan (1977), Singh and Qureshi (1977). But in this study stocking density and water quality parameters of all populations and hybrids were kept constant in all cages.

5.3.4 Percentage Weight Gain in Cages

Percentage weight gain of populations and hybrids were estimated. From the result it is found that highest growth rate among parent stock was observed in KR with 680.08 g and OR (582.92 g). Among parent populations AP showed least growth rate. Knibb *et al.* (1998) reported the variation in growth rate may be an effect of long term domestication. But in this study, AP was the domesticated strain and other two were wild prawns reared in hatchery for one generation. Hence in this study the difference in growth rate of different populations may be due to genetic variation. It was found that domesticated strains often perform better as they have adapted to culture environments channel catfish (Burnside *et al.*, 1975) and African Catfish (*Heterobranchus*

longifilis), (Nguenga *et al.*, 2000), unless they are highly inbred. Low growth rate of AP may be due to inbreeding depression.

Similar effects in growth rate were found in crosses also. Among hybrids KROR with 718.13 g showed faster growth than that of OR and KR. Growth rate pattern of three hybrids varied from parent populations. Percentage weight gain observed in KROR was 38.05% higher than that of KR and 135.21% higher than that of OR populations. Similar positive correlations in growth rate of hybrids were reported in many crustacean breeding studies. An earlier study reported that growth rates of crosses between Thai and Malaysian GFP strains performed better than pure Malaysian strain (Dobkin and Bailey, 1979). Hence cross breeding can produce productive outcomes potentially leading to hybrid vigour in the offspring. De Donato *et al.* (2005) reported that the gain in growth rate of *L. vannamei* by mass selection was 14.5% after 11 generations. From these results it is found that this species is suitable for selection. The significant additive genetic variance indicates potential for improving growth rate via application of artificial selection in *M. rosenbergii* (Thanh, 2010). According to Falconer and Mackay (1996), expression of heterosis in a cross between two particular lines or populations, depend on differences in gene frequency between the lines or populations and gene interactions. The directional dominance can reduce hybrid vigour due to the dominant nature of some loci in one direction, while some others are so in the opposite direction. While Fjalestad (2005) stated that the magnitude of heterosis depends on the level of genetic differentiation among parental populations.

But in APOR positive variation in weight gain was not much prominent as in the case of KROR. More over APKR showed a decrease in weight gain than that of parents. The negative effect on growth of APKR may be due to out breeding depression. From the study with genetic markers it is found that there was marked genetic difference between AP and other parent populations. The relative performance of crossbred and hybrid offspring in some instances could be either intermediate, or even inferior to their parental lines (Lutz, 2001). de

Bruyn *et al.* (2004b) showed that for two highly divergent clades of wild *M. rosenbergii* stocks in South East Asia, occurred naturally in the region. Combining highly divergent populations in a breeding program may increase the chance of out-breeding depression in their crosses and can also increase potential for contamination of wild gene pools if cultured 'hybrid' individuals escape to the wild. More over for *M. rosenbergii*, Malecha *et al.* (1984) found that maternal effects were only a minor contributor to performance of juvenile prawns. This finding is true in the case of APOR and APKR because growth performance was poor for APKR and APOR compared to KROR. Both in APKR and APOR paternal contribution from AP and maternal from KR and OR, where KR and OR populations showed superior growth parameters than AP population. From the growth study of parental population AP showed poor performance than that of KR and OR. Hence minor maternal contribution was transferred to the hybrids from the parents. That may also be one of the reasons for exhibitence of low growth of APKR and APOR. In contrast, Meewap *et al.* (1994) reported that *M. rosenbergii* sires and dams had a large influence on relative growth, of which, sires influenced all growth traits until 15 weeks in culture while dams influenced body length and weight until 23 weeks after metamorphosis. In this study sire and dam effect was not studied.

Coman *et al.* (2002) examined genotype-environment interactions on survival and growth in *M. japonicus* and found that the interactions for both traits were significant when differences in temperature were larger than 5^0C. But influence of such environmental factors has no role in these hybrids, because these populations and hybrids were developed under identical conditions in order to reduce the environmental effect on traits.

Age differences during stocking have been reported to result in differences in growth and survival in common carp (Dunham, 2004). Age differences among different hybrids during stocking were due to the variation in larval rearing periods of crosses. But, no such evidence for the effect of age or initial size at stocking effect on harvest body weight was observed in prawns

in this study. Shortest larval rearing period was found for KROR, which stocked first. But growth rate was lowest for this hybrid. Longest larval period was for APOR and the difference in stocking period was only 19 days. Hence the difference in weight gain here can be related to parental trait. Both AP hybrids showed lower growth as in the case parent population. Similar findings were reported in many previous studies of fish and shrimps (Palada-de Vera and Eknath, 1993; Bentsen *et al.*, 1998; Goyard *et al.*, 2002; Hussain *et al.*, 2002; Maluwa and Gjerde, 2006).

5.3.5 Percentage Morphotypes

After pond culture the adult prawns were sorted in to blue clawed males, orange clawed males, small males and females. From the data it was found that there was no significant variation in number of BC male in various populations and hybrids. Maximum OC males were found in AP followed by KROR. Orange clawed males have high growth potential. Highest small males were reported in OR and KR and in hybrids APKR. The highest percentage of female prawns was in KROR and lowest in OR but there were no significant variations. From the result it is clear that after pond culture the percentage of BC and FM were almost similar in all populations and hybrids. But slight variations in OC and SM were found among populations. In the initial phase of culture, differential growth was more prominent among populations. Availability of sufficient space, shelter and adequate feed may be one of the reasons for uniformity in morphotypes. In the field, stunting may occur as a temporary response when prawns are entrapped in a closed water body (Karplus, 2003). Introduction of shelters and partitions in the culture system help to reduce contact between SM and BC (Tidwell *et al.*, 1998). Size variation has both a genetic and environment component, but heritability of size has been found to be a sexually dimorphic trait (Malecha *et al.*, 1984).

Sun *et al.* (2000) reported that there was a substantial variation in androgenic gland proteins in PAGE (Polyacrylamide gel electrophoresis) study

of different morphotypes of prawns, indicating that the variation in cellular morphology of male morphotypes had genetic and social associations. The finding of Ranjeet and Kurup (2002a, b) supports this result.

5.3.6 Head –Tail Ratio

The data on head-tail weight ratio of different populations and their hybrids were estimated, which showed a significantly low ratio for OR, KROR and APOR. But KR showed an intermediate value and AP showed a higher value for head- tail ratio. The ratio of APKR was same as that of KR. The results show clearly that variation in the genetic background among the *M. rosenbergii* strains influenced head- tail ratio performance of purebred strains and its cross combinations.

5.4 HERITABILITY OF WEIGHT

Heritability of growth among full sib families of three hybrids of *M.rosenbergii* was estimated. From the result it is found that heritability of hybrid KROR was 0.745 and APOR 0.161 and APKR, 0. From these values it is clear that heritability was maximum for KROR hybrids. The high estimate of heritability for harvested weight in the KROR indicates the potential for rapid improvement of the population through selective breeding. Studies of heritability estimates were conducted by many workers in *M.rosenbergii*. Meewan (1991) estimated the heritability of growth among full and half sib families of freshwater prawn, *M. rosenbergii* and reported an estimate of 0.40±0.22 on paternal, maternal and full sib analysis. Uraiwan *et al.* (2003) found during the selective breeding of *M.rosenbergii* that there is a significant improvement in length and weight than control and an average heritability for length and weight was 0.38 and 0.22.

In contradictory to this result, cross between AP and KR (APKR) did not show heritability. Rattikansukha (1993) also reported similar results in interspecific hybridization of *M. rosenbergii* from two rivers, reciprocal cross and hybrid did not exhibit the heterosis.

The precision of heritability estimation is known to be affected by a large number of factors such as breeding design, type of relatives used, number and size of the families, family rearing approach and the method of analysis (Falconer and Mackay, 1996). The study, suffers from certain limitations, the most important of which is a relatively small number of families selected for estimating heritability leading to the moderately wide confidence intervals for the estimates may cause the variation between sets that was observed was large.

Summary

6. SUMMARY

Genetic characterization of Andhra Pradesh (AP), Orissa (OR) and Kerala (KR) stocks of *Macrobrachium rosenbergii* were done with RAPD and Microstellite markers. Marker analysis showed variation between the stocks.

Different phenotypic characters of larvae of the three stocks such as egg diameter, hatch fecundity, larval rearing period, survival rate, larval length and stress tolerance towards temperature, salinity, formaldehyde and ammonia were estimated and compared.

Economically important traits of juvenile and adult prawns such as viral and bacterial disease tolerance (challenge study), flesh quality (carcass proximate analysis), growth rate, survival rate, percentage morphotypes, and head -tail ratio were estimated and compared.

Large healthy prawns of the three stocks were selected from families showing better performance. Breeding experiment was conducted to produce six crosses viz. Andhra Pradesh (male)×Orissa (female)- APOR; Orissa (male) × Andhra Pradesh (female) -ORAP; Andhra Pradesh (male) × Kerala (female)- APKR; Kerala (male) × Andhra Pradesh (female)- KRAP; Orissa (male) × Kerala (female) –ORKR and Kerala (male) × Orissa (female) -KROR.

Different phenotypic characters and economically important traits of F1 generations of hybrids were also studied. Estimation and comparison of heritability of weight of three better performing hybrids were done.

Water quality parameters were checked during the experiment and were found to be within optimum levels for the growth of *M. rosenbergii*. All the stocks and hybrids were maintained under identical condition to reduce the effect of environmental factors.

Among the parent stocks better larval and trait performance were found for the KR stock. Among hybrids KROR exhibited similar or better performance than KR in all the trials. Specific cross combinations showed

indication for heterosis and the influence of direction of reciprocal crosses on phenotypic characters.

From the study it is established that there is genetic variation between stocks and this could be exploited strategically in future breeding programs.

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CHARACTERIZATION OF DIFFERENT STOCKS OF *MACROBRACHIUM ROSENBERGII* AND DEVELOPMENT OF GENETICALLY IMPROVED STRAIN THROUGH SELECTIVE BREEDING

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ABSTRACT OF THE THESIS

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ABSTRACT

Giant freshwater prawn *Macrobrachium rosenbergii* (de Man, 1879) is an important species suitable for culture in inland and low saline coastal water bodies. Information on the genetic structure and variation among cultured species are essential for genetic approaches to optimize conservation strategies and breeding programmes for improving stocks. DNA based markers can be used to assess more precisely the genetic variations in the entire genome. Natural populations of *M. rosenbergii* inhabiting the rivers in different geographical areas of India show wide variation in morphological traits and economic characters. The present work envisages studying the genetic diversity of wild populations of *M. rosenbergii* collected from different parts of India through phenotypic characters and molecular marker studies. It also aims to develop a superior strain by combining the desirable characters through selective breeding.

In this study genetic characterization of Andhra Pradesh (AP), Orissa (OR) and Kerala (KR) stocks of *Macrobrachium rosenbergii* were done with RAPD and Microstellite markers. Marker analysis showed variation between stocks and AP showed more genetic distance from OR and KR populations. Moreover KR stock from Achankoil River showed slight similarity with AP.

Phenotypic trait analysis was done with three stocks. Egg diameter, larval hatch fecundity, larval rearing period, survival rate and larval length were estimated. Highest egg diameter and hatch fecundity were shown by OR and KR populations. AP showed minimum larval rearing period and maximum survival rate. Maximum larval length was for KR.

Stress tolerance studies were conducted in V and X stage of *M.rosenbergii* larvae with temperature, salinity, formaldehyde and ammonia to estimate the relative differences in the ability of different populations to withstand stressed conditions. Among base population KR population showed highest tolerance towards temperature, salinity, formaldehyde and ammonia.

Stage wise tolerance variations also found in all populations. X stage showed more tolerance to all stressors except ammonia.

Comparison of other economically important parameters such as disease resistance, quality of flesh, percentage survival, growth rate, percentage morphotypes and head tail ratio were also done. Disease resistance was tested with white tail virus and bacteria (*Enterococcus aerogenes)* by challenge study. In both studies KR population exhibited better tolerance than other populations. Prophenoloxidase activity and total haemocyte count was also estimated in infected prawns to check the immune response. Here again KR showed higher activity and count. Flesh quality of three populations was estimated by carcass proximate analysis. In this study, no variation in body composition was shown by three stocks. Percentage survival rate and weight gain of three populations in cage were estimated. Maximum survival rate was found in AP and highest growth was found in KR stock. Analysis of percentage morphotypes of various populations showed the maximum OC for AP and SM for OR stock, with no significant variations in percentage BC and female prawns, head tail ratio, which was calculated to find out the yield of meat revealed the least ratio for KR.

Large healthy prawns of the three stocks were selected from families showing better performance. Breeding experiment was conducted to produce six crosses viz. Andhra Pradesh (male)×Orissa (female) APOR, Orissa (male) × Andhra Pradesh (female) ORAP, Andhra Pradesh (male) × Kerala (female) APKR, Kerala (male) × Andhra Pradesh (female) KRAP, Orissa (male) × Kerala (female) ORKR, Kerala (male) × Orissa (female) KROR.

Different phenotypic characters and economically important traits of F1 generations of hybrids were also studied. KROR showed better egg diameter, hatch fecundity, larval survival rate and larval length. But larval rearing period was minimum for APKR. KROR showed resistance to viral and bacterial diseases, which was similar to that of KR and better than other hybrids. In cage culture APOR showed better survival rate, while growth rate was

significantly higher for KROR, which was more than that of base populations. Analysis of percentage morphotypes showed a similar pattern as that of base populations. Lower head -tail weight ratio was shown by KROR, which was lower than that of KR.

Estimation and comparison of heritability of weight of three better performing hybrids were done. From the data it was found that maximum heritability of weight was for KROR (.75) followed by APKR (.161) and APKR exhibited no heritability.