REVIEW OF LITERATURE
II. REVIEW OF LITERATURE

Genetic studies are concerned with variation across a wide range of spatial and temporal scales from within individuals, among individuals within a population, among populations, to among species and higher level taxa. Different processes may operate at these different levels. Molecular genetics is principally concerned with the processes of development, mutation and the transfer of genes from one generation to the next. Population genetics refers to the distribution of genetic variation among individuals within a population, and among populations within a species.

Systematics is the study of relationships among species. Microevolution deals with changes seen within the species. Macroevolution is speciation and higher level divergence. The ideal marker to examine genetic variation within an individual requires a technique that can distinguish single base pair changes. Studies to find variants causing certain traits need markers spread throughout the genome. Species diagnosis would ideally use a marker that is invariant within a species but distinguishes it from all other species.

2.1. Difference between molecular and other markers

Markers are heritable variable traits that indicate the underlying genetic differences between individuals, populations, species or higher taxa. Non-molecular markers have some serious limitations; their inheritance can only be proven by controlled breeding trials i.e. non-mendelian inheritance; a trait in one group may not be present in other groups, making comparison between groups impossible; they may not reflect actual genetic variation due to phenotypic plasticity or the same character state may be the result of analogous traits. Morphological and ecological traits should not be disregarded since they reflect the results of
selection and there are examples of clear morphological and behavioural differentiation without genetic differentiation at neutral loci (Bensch et al., 1999).

Although molecular markers have been available for several decades, their use has grown very rapidly in the last few years. The variety of methods available, including polymerase chain reaction (PCR) leads to the explosion of information in all areas of genetics in recent years. The application of genetic markers has allowed rapid progress in aquaculture investigations of parentage assignments, genetic variability and inbreeding, species and strain identification and the construction of high-resolution linkage maps for aquaculture species (Liu and Cordes, 2004). Molecular markers could provide a method to assist the selection of individuals in a prawn-breeding programme, which would facilitate the domestication of species. Several molecular marker studies have demonstrated the ability to determine parentage, analyze pedigree and identify species (Herbinger et al., 1995; Perez-Enriquez et al., 1999; Sugaya et al., 2002; Chan, 2003; Hara and Sekino, 2003; Jackson et al., 2003; Castro et al., 2004; McDonald et al., 2004; Sekino et al., 2004; Vandeputte et al., 2004). The development of highly polymorphic genetic markers such as minisatellites and microsatellites has provided an essential tool for identifying parentage relationships among individuals and obtaining pedigree information in aquaculture selection programs (O'Reilly and Wright, 1995; Ferguson and Danzmann, 1998).

2.2. Selection criteria for the marker of choice

Different molecular markers provide genetic information, which is suited to only a certain range of questions at a particular scale (Waycott, 1998; Shaw et al., 1999; Sole et al., 1999). A very fine scale marker can detect and quantify differences between individuals but it becomes useless when applied to members of different species. Different markers may reveal
different patterns because they detect variability at different scales (Raybould et al., 1999). For example, many allozyme studies on marine fish have failed to detect any geographic structuring, although more rapidly evolving markers have in some cases revealed fine scale structure (Neigel, 1994). It is likely that each evolutionary or genetic process may have different effects on each type of marker. For example, the effective population size for mitochondrial DNA is quarter of that of nuclear markers; therefore any processes that are population size dependent will vary in their effect on mtDNA versus nuclear regions. Hence it is essential to choose a technique, which is appropriate to the question being asked (Yakubu et al., 1999).

There is a range of marker types available. They all have different characteristics such as cost, ease of use and type of variation detected. There is no perfect marker that can be used to answer all questions. The choice of marker to use depends not only on considerations of cost and ease-of-use, but most importantly on the questions for which answers are being sought (Yakubu et al., 1999). Levels of variation for any marker are in large part based on the rate and mode of mutation relative to the effects of genetic drift (Scribner et al., 1994).

2.3. Population genetic models and processes

The ideal population in a classic model consist of diploid sexually reproducing individuals, infinite in size (no stochastic effects), with no selection, migration, mutation or overlapping generations and perform random mating (Weir, 1996). For this ideal population, the frequency of each allele remains constant over time and genotype frequencies in one generation can be predicted from allelic frequencies in the previous generation (Hardy-Weinberg Equilibrium). Population genetic models attempt to describe the effects of one or a few processes that violate the assumptions of an ideal population.
2.3.1. Genetic drift

In a population of finite size, allelic frequencies will vary from generation to generation as a result of random sampling of gametes. This process is called genetic drift. When the allele frequencies drift to zero, those alleles get extinct from the population. This shows that two populations originated from the same ancestral population, if completely isolated for a long period, by genetic drift alone to have different allelic frequencies and even have fixed allele differences. Gene flow will prevent the populations from drifting apart, provided there must not be a high degree of isolation (Hedrick, 2000).

In an expanding population, the probability of occurring any drift is greatly reduced, as there is less likelihood of loss of any alleles. On the other hand, a temporary dramatic decrease in population size, called bottleneck, greatly increases the probability of allele loss. Similarly, the founder effect occurs when a new population is started by a very few individuals and thus has few alleles. The resultant low diversity can have critical implications if variation is low enough to prevent adaptations to new challenges (Hedrick, 2000).

2.3.2. Mutation and mating

Mutations may occur throughout the genome; however the rate of incorporation of mutations differs greatly in different regions of the genome. Coding regions are in general less variable than non-coding regions, presumably due to selection against changes that cause the protein less functional than the original version. Insertions and/or deletions in coding regions may cause frame shift mutations that can completely change the rest of the protein amino acid sequence. Some nucleotide substitutions, however, do not cause changes in amino acids due to
redundancy of the genetic code. These are called silent mutations or synonymous mutations (Di Rienzo et al., 1994).

All genetic variation first originates from mutations. It is the occurrence and subsequent persistence and the spread of these mutations that creates the variation, which allows evolution to occur. Breeding systems range from asexual reproduction to strict sexual mating. Even among the strict sexually reproducing species assertive mating can occur, or if there is unrecognised sub-population structure within a sample this can lead to an apparent deviation from Hardy Weinberg Equilibrium (HWE) even though within the sub-populations there is random mating. This is called Wahlund effect and is revealed as an excess of homozygotes in a strictly sexual species. While collecting such samples care must be taken to ensure that individuals are from the same population and collected from the same location and at the same time (Hedrick, 2000).

2.3.3. Gene flow

Some individuals may leave the population and produce offspring in another population. The level of gene flow has significant consequences for the evolution of populations and thus species. This high gene flow allows the spread of new alleles and reduces the genetic drifting apart of populations. The process generally slows down the local adaptation, but there are circumstances where gene flow may enhance local adaptation. There are three distinct indirect methods for estimating gene flow from observed genetic variation; they are the estimation from $F_{ST}$ analogs, private alleles and coalescent analyses (Slatkin and Maddison, 1989; Hudson et al., 1992).
2.3.4. 1. Migration and gene flow

A migrant must disperse, breed and successfully pass on its genes in order to cause gene flow. A common rule of thumb is that gene flow greater than one individual per generation is sufficient to prevent genetic differentiation of two populations through random drift. As each gene may have different critical levels due to different levels of selection, and the gene flow will be sufficient to prevent fixation of alternative alleles. This does not imply that allelic frequencies will be the same in both the populations under consideration (Allendorf and Phelps, 1981).

Gene flow is a unifying force that prevents populations from diverging. Gene flow breaks down the geographical or other boundaries that could otherwise isolate populations. As a result of isolation between populations, and the consequent limitations in exchange of genes, we expect that populations will diverge by genetic drift or as a result of selection for alleles that adapt each population to its local niche (Engel et al., 2004). But if gene flow occurs at a sufficiently high level, then otherwise isolated populations will not diverge genetically. Instead, they become united and evolve as a single evolutionary unit. Gene flow moves dominant or virulence alleles into new populations. Gene flow thus introduces new alleles that can displace old alleles, if they are better adapted to the current host. The population subdivision that results from genetic drift can be overcome by gene flow (Hedrick, 2000). If enough individuals are exchanged between two populations that are experiencing independent genetic drift, then the drifting populations become genetically linked and population subdivision will not occur.

2.3.4.2. Evaluation of migration and gene flow
Understanding migration and gene flow between populations is one of the most important aspects of population genetics, and is vital when investigating conservation genetics, hybridization, and disease genetics. Estimates of divergence between populations (e.g. using $F_{ST}$ or similar statistics, or coalescent methods) are useful for understanding historical gene flow and relationships between populations, but newer methods attempt to quantify the level of recent migration (Wilson and Rannala, 2003) or identify the parents of particular individuals (Jones and Ardren, 2003) using more sophisticated algorithms. One way to determine recent migration and gene flow history is to perform assignment tests.

There are several types of assignment methods available to researchers. Assignment tests based on likelihood algorithms are the most popular as they can estimate population structure without a prior knowledge of population limits (Pritchard et al., 2000; Corander et al., 2003; Falush et al., 2003; Manel et al., 2005). Bayesian assignments tests have received the greatest attention over the past few years, with clustering-based methods by far the most widely used. Programs like STRUCTURE (Pritchard et al., 2000), BAPS (Corander et al., 2003), and STRUCTURAMA (Huelsenbeck and Andolfatto, 2007) can all assign individuals to a cluster based on a probabilistic model, each with slightly different underlying assumptions and different methods of searching parameter space. The assignment method developed by Rannala and Mountain (1997), implemented in either GeneClass2 (Piry et al., 2004) or BayesAss+ (Wilson and Rannala, 2003), can be used to estimate the frequency of migrants within known populations. A major advantage of this method over other clustering assignment tests is that they provide a posterior probability of each individual’s migration ancestry. These various methods can complement each other, with clustering assignment tests used to
determine the appropriate population substructuring followed by analyses using assignment methods to estimate recent migration between those demes.

2.3.5. Selection

Most genetic models assume that the genetic markers are selectively neutral. Genetic markers provide useful information because they are variable within and/or among populations or species. In order to explain this variation in terms of population genetic processes, it is necessary to make certain assumptions about how the variation arose.

The infinite allele model was originally developed for allozyme electrophoresis and restriction sites. It assumes that each new version of allele is equidistant from each other version (Tajima, 1996). Sequence data and restriction site analysis ideally use this model. These types of data can potentially reveal the phylogenetic history of the sequence. It cannot be used, when mutation rate varies substantially among sites (Tajima, 1996).

2. 4. Measures of diversity

Measures of diversity indicate the extent of genetic variation in a population. There is a large number of diversity measures applicable to different types of markers: percent of variable loci; average number of substitutions/site; heterozygosity, frequency of private alleles (Nei, 1987).

Hierarchical analysis finds the distribution of the total genetic variation between hierarchical levels. All $F_{ST}$ analogs attempt to quantify the amount of total variation that is due to differences between populations. Most statistics that describe genetic differentiation from genetic markers (e.g., F-statistics) rely solely on allele identity information. This information is often used to infer phylogenetic relationships or to obtain indirect estimates of gene flow.
2.5. Molecular genetic markers and their utilities

The utility of molecular genetic markers extends beyond mapping and fingerprinting experiments into population genetics, where allele frequencies rather than individuals serve as the focus of study. Allele frequency data are useful for studying evolutionary relationships of closely related species or populations.

Microsatellite loci are the loci that vary in the number of repeats of a simple DNA sequence and are becoming commonly used in the analysis of natural populations. Microsatellite loci are often highly polymorphic and relatively easy to survey and hence offer a path for greater understanding of population structure. Microsatellite loci are typically characterized by high mutation rates and hence a high level of polymorphism. The mutation process, that causes preferentially stepwise changes in the number of repeats and thus allele size in a microsatellite locus (Arcot et al., 1995, Zhu et al., 2000). Hence, the difference in size between two different alleles might be informative. The larger the difference, the higher the number of mutation events (thus time lapse) is expected to have occurred since common ancestry. There is thus a “memory” of past mutation events (Levinson and Gutman, 1987).

Two alleles are homoplasic when they are ‘identical in state’ (IIS), though not ‘identical by descent’ (IBD). Homoplasy depends strongly on the model of molecular evolution considered. Among the most widely used models, both the stepwise mutation model (SMM) (Ohta and Kimura, 1973, Valdes et al., 1993) and the K-alleles model (KAM) (Kimura, 1968) generate some homoplasy, while the infinitealleles model (IAM) (Crow and Kimura, 1964) does not. Homoplasy has important consequences in population genetics, because it affects how population structure may be interpreted. However, the consequences of
homoplasy on the parameters used to describe population structure are difficult to predict (Rousset, 1996).

As microsatellites may evolve in a stepwise fashion, and fit either the SMM or the two-phase model (TPM), homoplasy is expected at microsatellite loci (Freimer and Slatkin, 1996; Jarne and Lagoda, 1996). Variability at microsatellite loci is generally analyzed as size differences between electromorphs, i.e., stretches of amplified DNA at a particular locus migrating at the same speed in an electric field. Electromorphs are therefore IIS. However, it is sometimes possible to distinguish between electromorphs through sequencing, as a given electromorph may hide a set of different sequences (hereafter referred to as alleles). Part of homoplasy can therefore be uncovered, and this is referred to as size homoplasy. All the identical sequences are IIS but not necessarily IBD. Size homoplasy at microsatellite loci may also arise as a consequence of the variation in flanking regions, particularly among species (Grimaldi and Crouau-Roy, 1997).

2.6. Population management

Proper management of the population begins with the acquisition of the stock and this is a crucial step, whatever the goal may be. The acquisition of a stock is difficult and expensive and should be considered the single most important aspect of broodstock management because this act determines the maximum amount of genetic variance and also determines how much inbreeding will be produced via future matings (Tave, 1999). The genetic size of the acquired population (\(Ne\)) will have the greatest effect on the probability of success in the fish culture experience. A “founder effect” (small genetic size) would be dramatic since it will produce inbreeding and genetic drift.
2.7. Inbreeding

Inbreeding is a more serious problem in fish and prawn farming than it is with livestock. Fish and prawn are highly fecund and there is a great temptation to spawn as few fish as possible in order to save money and labour. If inbreeding occurs, it can reach levels that cause growth rate or other production phenotypes to decrease significantly (Kincaid, 1976, 1983; Gjerde et al., 1983; Sbordoni et al., 1986; Su et al., 1996). In addition, the loss of genetic variation (genetic drift) can make future selective breeding programs ineffective. The selective breeding becomes important since genetics produces superior stocks of fish and raises the yearly yield significantly through selective breeding and crossbreeding programs (Bartley, 1998; Tave, 1999).

Constructing and maintaining pedigrees have proven difficult for both wild and captive fish and/or prawn populations. Parentage assignment using microsatellites has proven to be very useful in aquaculture. DNA microsatellites could be used for retrospective parental assignments and comparisons of viability of offspring with different parental origins without bias from unknown variations in laboratory systems or farm environments and, moreover, they make possible the estimation of relatedness between pairs of individuals with unknown origin (Estoup et al., 1998a; Norris et al., 2000; Jackson et al., 2003; Sekino et al., 2003; Borrell et al., 2004).

The level of inbreeding of an individual is usually measured by the inbreeding coefficient ‘f’ (the probability that two alleles at a locus are identical by descent in that individual, Gall, 1987). There is a close mathematical relationship between f and relatedness ‘r’; thus for non-inbred individuals: \( f = \frac{r_{FM}}{2} + \frac{r_{MF}}{2} \) where \( f \) is the inbreeding coefficient of the offspring, \( r_{FM} \) is relatedness between the female and the male and \( r_{MF} \) is relatedness
between the male and the female (Wilson, 1980; Hartl and Clark, 1989; Liautard and Sundstrom, 2005).

Inbreeding is a main issue in evolutionary and conservation biology because of its detrimental effects on fitness and its consequences upon population viability (Frankham, 1995). Mating between relatives leads to a reduction in individual fitness known as inbreeding depression, which is thought to be the consequence of the increased homozygosity through identity by descent at all loci across the genome (Charlesworth and Charlesworth, 1987; Ballou et al., 1995). Inbreeding depression could be the result of two different mechanisms. First, inbreeding can lead to the expression of recessive deleterious alleles when homozygosity increases (dominance hypothesis). Alternatively, heterozygotes may be fitter than either homozygote (overdominance hypothesis). Expression of deleterious recessive alleles is thought to be the main cause of inbreeding depression (Charlesworth and Charlesworth, 1999).

Imperfect pedigree information may lead to miscalculations of the inbreeding coefficient (Marshall et al., 2002; Markert et al., 2004; Pemberton, 2004, 2008). A common problem both in captive and wild populations is that the assignment of paternity on the basis of behavioural observations (copulations) may lead to mistakes when females mate promiscuously; extra-pair copulations are more difficult to detect because they tend to be less conspicuous, leading to errors when constructing pedigrees unless paternity is determined by molecular methods (O’Connor et al., 2006). Incomplete information may also make the pedigree inaccurate because unknown individuals are assumed to be unrelated (Marshall et al., 2002).

Constructing pedigrees requires detailed information, which is not always available, and in these cases inbreeding coefficients cannot be calculated. An alternative approach has been developed to test for inbreeding depression indirectly. If inbreeding causes
heterozygosity depletion, then measuring heterozygosity levels with molecular markers, may provide a good indication of inbreeding levels. Currently, microsatellites are frequently the markers of choice to estimate heterozygosity and different measures have been developed. These include: multilocus heterozygosity (MLH) and standardized heterozygosity (sMLH) (Coltman et al., 1999), internal relatedness (IR) (Amos et al., 2001), and heterozygosity by locus (HL) (Aparicio et al., 2006).

2.8. Polymorphic information content (PIC)

The PIC (Botstein et al., 1980) is a metric which characterizes how informative a genetic marker is; it was defined for measuring the usefulness of a co-dominant genetic marker to identify the allele, transmitted by a heterozygote parent which is affected from a genetic disease, whose expression is from the dominant allele. PIC is always smaller than heterozygosity, as some crossings among heterozygous individuals may not be informative. For a locus with k alleles, PIC can be calculated as:

$$\text{PIC} = 2 \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} p_i p_j (1 - p_i p_j)$$

where $p_i$ is the population frequency of the $i$-th allele and $k$ is the total number of alleles.

2.9. Genetic diversity and its effective maintenance

Genetic diversity is of great importance to the sustainability of populations (Hamrick et al., 1991). Genetic variation within populations can be lost through genetic drift (Allendorf et al., 1987), a process intensified when population size becomes small. Genetic variation among populations can be lost when previously restricted gene flow between populations is increased.
by stocking or removal of natural barriers, causing differentiation between populations to be lost as a result of the homogenization of two previously distinct entities (Altukhov and Salmenkova, 1987; Campton, 1987; Utter, 2003).

The primary two procedures for maintaining genetic diversity have been the establishment of *ex situ* gene banks and maintenance of *in situ* natural genetic resources. However, conservation of genetic diversity through the use of gene banks has been hindered by many factors including limited facilities, expense, and difficulties involved in the preservation of female gametes. While storage of sperm has been relatively straightforward, cryopreservation of eggs has been difficult due to their large sizes (Tiersch *et al.*, 1994). It is believed that sustainability of natural genetic resources is fundamentally important for the preservation of genetic diversities.

Among the molecular markers available in population genetics, microsatellites emerged as those with finest resolution for labelling of populations and individuals, due to their high variability, abundance, neutrality, codominance and unambiguously scoring of alleles (Tautz, 1989; Weber and May, 1989). Microsatellite genotyping has proved to be a powerful tool for accurate genetic assessment of population differentiation and pedigree tracing of hatchery populations from various fishery animals (Norris *et al.*, 2000; Neff, 2001; Sugaya *et al.*, 2002; Li *et al.*, 2003; Yu and Guo, 2005).

### 2.10. Importance of microsatellite DNA as a molecular marker

Microsatellites are also called simple sequence repeats (SSR), short tandem repeats (STR), or simple sequence length polymorphisms (SSLP) but microsatellites and simple sequences repeats (SSR) are now the most widely accepted denomination. Though some can be found in transcription units (Perry and Bousquet, 1998), they have no known function and
Microsatellites are powerful mapping tools due to their polymorphism, codominance and cross priming in different species and are also good candidates as anchor points within and across genetic maps. The use of these markers in fundamental research (genetic structure, gene flow studies, genome mapping and molecular phylogenetics) and applied research (marker-aided breeding, provenance testing, commercial certification) is now under way (Ellegren, 2000). Given the time and costs needed to identify such markers, potential cross-amplification of primers designed for a species between species and genera has to be extensively examined, although successful amplification tends to decrease with an increasing evolutionary distance.

SSRs are appropriate markers for describing patterns of intraspecific genetic diversity and assessing the evolutionary history of populations (Zhang and Hewitt, 2003) because they are usually highly polymorphic, multi-allelic, show codominant inheritance, and results can be analysed using straightforward statistics (Goldstein and Schlotterer, 1999). The use of SSR markers poses some problems in taxa exhibiting polyploidy, mainly because of the inability to
identify precisely the number of homologous alleles with these markers, and therefore to estimate other statistics such as heterozygosity (Trewick et al., 2002; Wilmhoff et al., 2003; Andreakis et al., 2007; Huang et al., 2008). Several methods have been proposed to render microsatellites suitable also in polyploid species (Samadi et al., 1999; Espinoza and Noor, 2002; Bruvo et al., 2004; Babaei et al., 2007). In all these methods, individual alleles for each locus are scored in a binary matrix and similarity indices developed for dominant markers such as amplified fragment length polymorphisms (AFLP) and restriction fragment length polymorphisms (RFLP) are applied. Here we score each locus amplification products as in a dominant marker system, and we make use of appropriate computer programs and statistical descriptors developed for dominant markers. On the basis of frequencies gathered from microsatellite alleles and multilocus genotypes, we infer robust population-level statistics in this species despite the disparities encountered in its ploidy level.

2.11. Measures of the microsatellite variation

Microsatellite polymorphism is based on the short sequences that are repeated in tandem at one or more places in the genome. Each location in a chromosome that contains core repeats may have different number \( n \) of copies of the repeat (Schlotterer and Tautz, 1992). This means that at any particular location, a microsatellite repeat has multiple allele in the population, where each allele different in its value of number. With this approach, each genome yield a different fragment size due to the differing number of core repeat, and therefore yields a distinct allele in the gel electrophoresis.

Alleles in natural populations usually differ in frequency from one allele to the next. The allele frequency of a prescribed allele among a group of individuals is defined as the proportion of all allele at the locus that are of the prescribed type. The frequency of any
prescribed allele in a sample is therefore equal to twice the number of genotypes homozygous for the allele (because each homozygote carries two copies of the allele), plus the number of genotypes heterozygous for the allele (because each heterozygote carries one copy), divided by two times the total number of individuals in the sample (Shriver et al., 1993).

The allele frequency in a population for diploid organism can be estimated the by equation below (Guo and Thompson, 1992). In generally, suppose that among ‘N’ individuals sampled from a population the numbers of ‘AA, Aa, and aa’ genotypes are ‘NAA, NAa, and Naa’ respectively. If p and q represent the allele frequency of ‘A and a’, respectively, with ‘p +q = 1’. The estimate of allele frequency in the population can be equated as

\[
\text{Allele frequency} = \frac{2N_{AA} + N_{Aa}}{2N}
\]

and the estimated sampling variance is

\[
[\text{Var}(p)] = \frac{(p)(1-p)}{2N}
\]

From the equation, N’AA’, N’aa’ were represented the number of homozygous at allele ‘A and a’, and NAa was represented the number of heterozygous, for such allele, N is the number of investigated individuals.

The genotype frequencies for a gene with two alleles can be deduced. Assume that genotype frequencies of AA, Aa and aa in the parental generation are D, H and R, respectively, where \(D+H+R = 1.0\). The allele frequencies of A and a are given by

\[
p = \frac{(2D+H)}{2} = D + H/2 \quad \text{and} \quad q = \frac{(2R + H)}{2} = R + H/2
\]

The new genotype frequencies are calculated as the sum of the cross products shown.

\[
D' = D^2 + 2DH/2 + H^2/4 = (D + H/2)^2 = p^2
\]
\[ H' = \frac{2DH}{2} + 2DR + \frac{H^2}{2} + 2HR/2 = 2(D + H/2)(R + H/2) = 2pq \]

\[ R' = \frac{H^2}{4} + 2HR/2 + R^2 = (R + H/2)^2 = q^2 \]

The new genotype frequencies \( P', Q' \) and \( R' \) simplify to:

\[
\begin{align*}
AA : D' &= p^2 \\
Aa : H' &= 2pq \\
Aa : R' &= q^2
\end{align*}
\]

a result known as the Hardy-Weinberg principle after Godfrey Hardy and Wilhelm Weinberg in 1908. The Hardy-Weinberg principle provides the foundation for many theoretical investigations in the population genetics. One of the most important implications emerges when we calculate the allele frequencies \( p' \) and \( q' \) of \( A \) and \( a \). the allele frequencies of \( A \) and \( a \) are (Hartl, 2000)

\[
\begin{align*}
p' &= \frac{(2D' + H')}{2} = \frac{(2p^2 + 2pq)}{2} = p(p + q) = p \\
q' &= \frac{(2R' + H')}{2} = \frac{(2q^2 + 2pq)}{2} = q(p + q) = q
\end{align*}
\]

Because the allele frequencies remain the same generation after generation, so do the genotype frequencies in the proportions \( p^2, 2pq \) and \( q^2 \), which are often called the Hardy-Weinberg equilibrium (HWE).

For Chi-square test \( (\chi^2) \) for HWE, the mere fact that observed genotype frequencies may happen to fit HWE cannot be taken as evidence that all of the assumptions in the model are valid. The principle is not very sensitive to certain kinds of departures from the assumptions, particularly those pertaining to a very large population size with no migration, mutation, or selection. On the other hand, the relative insensitivity departures from its assumptions gives the principle some robustness, because it implies the HWE can be valid to a first approximation even when one or more of the assumptions is violated.
The usual test for goodness of fit of observed data to HWE is a chi-square test. The test statistic is usually symbolized $\chi^2$, and under the hypothesis of HWE the $\chi^2$ has approximately a chi-square distribution. The value of $X^2$ is calculated as

$$\chi^2 = \sum \frac{(\text{obs} - \text{exp})^2}{\text{exp}}$$

For linkage disequilibrium, HWE, statistically, means that the alleles present at a locus are in random association with each other in the genotypes. It therefore may seem paradoxical that two gene, $A$ and $B$, present in the same population may each obey HWE individually, yet the allele $A$ and $B$ can remain in nonrandom association in the gametes that from each generation. The measure of linkage disequilibrium have a predefined range is

$$p = \frac{D}{\sqrt{pq_1q_2}}$$

The correlation between linkage the $A$ and $B$ alleles present in gametes and can range from $-1$ to $+1$. This measure has the convenient feature that the $\chi^2$ value for goodness of fit to the hypothesis that $D = 0$ is given by (Hartl, 2000).

$$\chi^2 = p^2 n$$

In $F$ coefficients, Wright (1965) developed an approach to partitioning the genetic variation in a subdivided population that provides an obvious description of differentiation. This approach consists of three different $F$ coefficients used to allocate the genetic variability to the total population level (T), subdivisions (S) and individuals (I). These three values, $F_{ST}$, $F_{IT}$, and $F_{IS}$ are interrelated so that

$$F_{ST} = F_{IT} - F_{IS} / 1 - F_{IS}$$
F_{ST} is a measure of the genetic differentiation over subpopulations and is always positive. F_{IT} and F_{IS} are measures of the deviation for Hardy-Weinberg proportions within subpopulations and in the total population, respectively, where positive value indicate a deficiency of heterozygotes, and negative values indicate an excess of heterozygotes (Hedrick, 2000).

2.12. Parentage analysis

Parentage analyses can provide a finer scale assignment test than those that assign individuals to clusters based on genotypes. More information is needed however, and this can vary depending on the level of sampling possible and/or the prior knowledge of relationships (Jones and Ardren, 2003). Exclusion based parentage analyses are the simplest form of parentage assignment, but require the most a priori information (Jones and Ardren, 2003). Categorical allocation of parentage can be used when exclusion-based analyses fail to exclude all but one potential parent (Meagher and Thompson, 1986). Fractional allocation is similar to categorical allocation, but it is allowed to assign only a fraction of the offspring to a potential parent (Devlin et al., 1988). Both the categorical and fractional methods use a likelihood-based approach where a log of the odds (LOD) score is used to statistically assign parentage. Both methods perform best with exhaustive or near-exhaustive sampling of all individuals. However, not only are exhaustive sampling difficult in highly mobile species, but completing this for multiple populations are prohibitively expensive and time-consuming in most systems. Even in botanical studies where the immobility of individuals makes complete sampling of reproducing individuals easier, there is often the issue of dormant individuals within the seed bank.
2.13. Cross species usage of microsatellite DNA markers

The widespread use of microsatellites has been limited by the fact that PCR primers require a high degree of homology to work, implying that novel species-specific markers would have to be isolated when starting the analysis of a new species (Steinkellner et al., 1997). Success in the cross-species amplification of any DNA sequence is inversely related to the evolutionary distance between the two species (Steinkellner et al., 1997). Hence, research on species relationships has increasingly focused on assessing the ability of SSR primers to amplify the same loci across different species and genera (Byrne et al., 1996; Katzir et al., 1996; Isagi and Suhandono, 1997; Smulders et al., 1997; Steinkellner et al., 1997; Witsenboer et al., 1997). The development of which remains a costly and lengthy process despite the continuing improvements in its efficiency (Zane et al., 2002). Transfer of primers (cross-species amplification) offered an alternative to de novo development in plants (Peakall et al., 1998, Rossetto, 2001), with transfer rates ranging from 90% within subgenera to 35% within the family, and potential polymorphism from 78 to 58%, respectively (Rossetto, 2001).

2.14. Linkage disequilibrium (LD)

The pattern and extent of non-random associations among polymorphic markers distributed over the genome are related to the evolutionary rate of population structure for a species (Tishkoff et al., 1996, 2001; Stephens et al., 2001; Ardlie et al., 2002; Weiss and Clark, 2002). One measure of such non-random associations, linkage disequilibrium (LD), is often affected by various evolutionary forces, such as selection, genetic drift, mutation, admixture, population structure, etc., which have operated in the population. For this reason, by estimating and testing for the extent and distribution of LD throughout the genome, the
evolution of population structure can be inferred (Tishkoff and Williams, 2002). LD analysis based on multiple markers can provide additional information about population structure by estimating the extent and distribution of non-random associations throughout the genome (Stephens et al., 2001; Ardlie et al., 2002; Dawson et al., 2002). For a random-mating population, the LD between two markers decays with generation in a proportion depending on the recombination fraction between the markers (Lynch and Walsh, 1998). Thus, by comparing the rate of LD decay over genetic distances, the evolutionary history of a population can be inferred (Dawson et al., 2002; Gabriel et al., 2002; Tishkoff and Williams, 2002). Also, the rate of the LD decay as a function of generation has established a fundamental principle for the high-resolution mapping of complex traits in a population (Rafalski and Morgante, 2004).

2.15. Null alleles

Null alleles have posed recurring challenges for population genetics following the introduction and application of each new method of molecular assay (including serological typing, protein electrophoresis, RFLPs, and microsatellites). One of the earliest examples involved the human ABO blood group system, wherein the O allele is a null allele that produces no phenotype (i.e., is masked by the presence of the A or B alleles, which are codominant to each other). For the special ABO case, formulae have been developed for estimating the frequency of the O allele (Yasuda and Kimura, 1968) as well as for calculating the average paternity exclusion probability (Weir, 1996). By definition, a microsatellite null allele is any allele at a microsatellite locus that consistently fails to amplify to detected levels via the polymerase chain reaction (PCR).
Null alleles can also be generated via differential amplification of size-variant alleles (Wattier et al., 1998). Due to the competitive nature of PCR, alleles of short length often amplify more efficiently than larger ones, such that only the smaller of two alleles might be detected from a heterozygous individual. Null alleles caused by differential amplification are sometimes termed ‘partial nulls’ because they can often be made visible by loading more samples or by adjusting contrast. A third source of null alleles involves PCR failure due to inconsistent DNA template quality or low template quantity. These problems are insidious because in some cases only one or a few loci (or alleles) fail to amplify, whereas others amplify with relative ease from the same DNA preparation (Gagneux et al., 1997; Garcia de Leon et al., 1998). When DNA template at a locus is poor in some specimens but not others, the poor samples may appear ‘homozygous’ rather than heterozygous for the null allele.

Apart from these primary causes of ‘bona fide’ null alleles, several population genetic phenomena might give the false impression that microsatellite null alleles are present in a given study. Biological factors such as Wahlund effect or inbreeding, for example, can cause significant heterozygote deficits relative to Hardy–Weinberg equilibrium (HWE) that might be misconstrued as evidence for null alleles (Chakraborty et al., 1992). However, proper multilocus analysis can normally distinguish these causes because such population genetic factors should register more or less concordantly across loci, whereas the effects of null alleles are locus-specific.

2.16. Marker–assisted selection

Mapping disease-resistance genes is a challenge in many organisms, because often disease-resistance cannot be quantified for a given genotype. The identification and mapping of disease-resistance genes or quantitative trait loci (QTLs) may provide valuable information
and tools for marker-assisted selection. Marker-assisted selection is particularly useful for the
development of disease resistant prawns because breeding decisions are sometimes made in
the absence of disease-exposure (Guo, 2004). One of the prerequisite for QTL mapping is the
availability of a large number of genetic markers. Two types of markers, microsatellites (MS)
and amplified fragment length polymorphisms (AFLP), are commonly used for linkage and
QTL mapping (Lewis et al., 1990). MS markers are excellent markers for QTL mapping
because of their high levels of polymorphism and co-dominant nature. MS are also expensive
to develop and use (Brown et al., 2000; Reece et al., 2004).

Marker assisted selection (MAS) is based on the concept that, it is possible to infer the
presence of a gene from the presence of a marker tightly linked to the gene. Strategies to find
markers tightly linked to the target gene are similar to those that are used for fine-QTL
mapping. Once a tight linkage is found between a molecular marker and a gene of interest, the
inheritance of the gene can be traced in breeding programme (Chistiakov et al., 2006). For a
successful MAS programme, clearly identified and quantified phenotypes are a prerequisite.
Microsatellite markers, which are linked to the target QTLs, are useful in the preliminary
stages of parents for crossing and thereby subsequent genetic characterization of the progeny.
There are not yet large-scale fish breeding programme using MAS. However, the current
status of genetic information and advanced genomic tools available for rainbow trout, Atlantic
salmon and channel catfish provide an opportunity for the successful application of the DNA
marker-based technology to selective breeding in these species (Chistiakov et al., 2006).

AFLPs are anonymous and dominant markers that are less transferable and informative
than microsatellites, but they can be effectively used in backcrosses as co-dominant markers.
Their poor transferability is compensated by the large number of markers that can be quickly
developed without prior knowledge of DNA sequences. AFLP markers have been widely used for QTL mapping and breeding in plants (Jin et al., 1998; Goodwin et al., 2003; Bai et al., 1999; Hartl et al., 1999; Altinkut et al., 2003), as well as in aquatic animals (Jackson et al., 1998; Palti et al., 1999; 2001 and 2002; Streelman and Kocher, 2002; Shirak et al., 2002; Cnaani et al., 2003). AFLPs have been shown to be effective in linkage mapping in oysters (Yu and Guo, 2003; Li and Guo, 2004). Because of the nature of AFLP markers, markers segregating through the female cannot be mapped to the male map for comparison and consolidation.

Another requirement for QTL mapping is availability of reference families where QTLs are well defined and segregating. There is no highly inbred and disease resistant stock available for making reference crosses in the giant freshwater prawn. On the other hand, high levels of variability in the disease-resistant and wild stocks may provide sufficient segregation of disease-resistance QTLs. Another limitation is that resistance to some diseases can only be measured by survival. Disease-resistance QTLs can only be identified by markers, which show significant frequency shifts after disease-inflicted mortalities.

2.17. Importance of effective population size

The success of hatchery breeding programmes is related strongly to the effective population sizes that are used for founding new generations (Pante et al., 2001; Koljonen et al., 2002). The most widely used method in hatcheries traditionally has been to pick out a few individuals with favourable characteristics, and use those individuals as founders to produce thousands of offspring. This method involves a bottleneck phenomenon effect. Consequently, the hatchery produced broodstocks face the risk of inbreeding due to elevated levels of relatedness among individuals and, therefore, loss of genetic variation. Pante et al., (2001)
showed that the genetic composition of broodstocks of rainbow trout, *Oncorhynchus mykiss*, varied depending on number of founders and the methods that were used. The size of the founding population is an important factor in the overall level of genetic variability over time. A small founding population means lower levels of genetic variation, which then decreases by drift over time. This reflects an important issue that must be considered in establishing hatcheries, namely founder history. If a few founder individuals are used to produce offspring and a few of these offspring are used later as founder individuals themselves, this will create an even narrower bottleneck compared to the situation where few founder individuals are used but are replaced continuously with wild-caught individuals. Basic population genetic theory predicts that heterozygosity decreases about 54% after 15 generations with an effective population size of only 10 (using the relationship $H(t) = (1 - 1/2*Ne)H(0)$, where $H$ is heterozygosity at time $t$ and zero (start)). Increasing effective population size to 30 individuals results in an expected decrease of heterozygosity of 22% after 15 generations, and with 50 individuals the reduction is only 14%. Thus, a rather moderate increase in effective population size at founding pays off quickly in terms of lower rate of loss of genetic variability.

2.18. Fitness difference between natural and hatchery reared populations

The impact of stocking on natural populations has been discussed widely and investigated in many ways; the studies done during the last decade have mostly been conducted using a genetic approach. A number of these studies have shown differences between wild- and hatchery-produced fish that could lower the fitness of wild individuals in the case of introgression (Reisenbichler and Rubin, 1999). The situation in hatcheries traditionally has been that a limited number of individuals with favourable characteristics have been used to produce thousands of offspring (Wedekind, 2002). One male can, for instance, be
used to fertilise a number of females in several generations, which inevitably leads to that same male fertilising his own offspring. This will of course strongly contribute to the depletion of genetic diversity and thereby to inbreeding. The genetically depleted individuals produced in hatcheries are then released into the wild. The effect of these individuals on wild populations can be numerous: depletion of genetic diversity, contamination of diseases and increased competition (Bohlin et al., 2002; Heggenes et al., 2002). As salmonid fishes are characterized by the existence of a number of local populations that exhibit a large degree of differentiation between them (Hindar et al., 1991; Estoup et al., 1998b), the genetic backgrounds of stocked-individuals are important to consider when released into the wild. The major reasons for loss of genetic variation in hatchery populations most often are bottlenecks and small effective population sizes, due to inappropriate mating designs. It is therefore of great importance that hatchery methods are optimised with regard to mating designs (number of breeders, relatedness of breeders, etc.) when it comes to preserving genetic variation and passing that variation on to the next generation.

2.19. Asymptomatic carrier organisms of shrimp virus

Many of the wild aquatic crustaceans such as pest crabs, mud crabs, pest prawns, insects, lobsters, shrimps, mantis shrimp and freshwater prawn harbour WSSV (Lo et al., 1996; Otta et al., 1999; Hossain et al., 2001a, 2001b). These crustaceans act as reservoirs and may transmit the virus to healthy shrimps during culture (Wang et al., 1998). Good management practices have been proposed as the only approach to significantly reduce the heavy mortality, as there is no cure for viral diseases. Till recently, diagnostic procedures for viral disease were largely dependent upon classical methods such as history, clinical signs and histological examinations that were often limited by low sensitivity (Durand et al., 1996). This
has been overcome by the development of molecular methods for rapid disease diagnosis (Lightner and Redman, 1998). Apart from rapidity, molecular methods also allow the detection of asymptomatic carriers (Otta et al., 1999; Nadala and Loh, 2000), which harbour low levels of virus load.

Many carrier animals that are common in prawn culture area transmit the virus to penaeid culture system with intake of water (Chakraborthy et al., 2002). Crustacean carriers, which enter prawn ponds, may transmit WSSV and when they die is likely to be eaten by shrimps due to their cannibalistic nature. Birds may mechanically transmit the virus between ponds by releasing captured shrimps over neighbouring ponds. Within the culture system WSSV is transmitted by cannibalism of moribund prawns and carcasses or via contaminated water (Chang et al., 1996). MBV is transmitted by ingestion of free virus and cannibalism. It is also believed to be transmitted vertically from broodstock to offspring. HPV is transmitted vertically from brood stock to progeny and horizontal during the post larval stages (Brock and Lightner, 1990). In two studies on captured brood stock specimens (Flegel et al., 1997), none showed characteristic histopathology of HPV. This suggests that the virus may not originate with the brood stock but with some other carrier in the cultivation system. Successful experimental infections by oral challenge in post larvae of the black tiger shrimp, *P. monodon* have recently been reported (Catap et al., 2003). Catap and Travina (2005) have also reported successful horizontal transmission of HPV in *P. monodon* post larvae.

### 2.20. Important crustacean viral diseases of significance in India

The practise of transporting shrimp stocks between facilities and/or different geographic regions has resulted in the introduction of many known penaeid shrimp viruses to regions where they may not have previously existed. Whether or not these introduced viruses
have escaped the culture facilities to which they have been introduced and have become established in local wild penaeid stocks is not known. Evaluation of non-native penaeids by the rapidly growing shrimp culture industry is an essential component to the future growth and development of that industry. Prevention of such exotic pathogen introduction is dependent upon the use of quarantine, certification and inspection policies, and procedures that are supported by reliable diagnostic tests. Since there is no known treatment for viral diseases, the major strategy for disease management is avoidance. However, in practice this is very difficult. In Asia, the devastating mortalities caused by WSSV have led to development of strategies such as PCR screening of brood stock before spawning and PCR screening of larvae before stocking to avoid the entry of virus into aquaculture system. But, this has led to only partial success because WSSV has a broad host range (Lo et al., 1996, Hossain et al., 2001a, 2001b) and can survive in a number of carrier animals that can be a source of virus for the ponds. Further, WSSV survives in water up to 20 days. Hence, avoidance of pathogens can be done through selection of specific pathogen free broodstock, exclusion of carrier animals in culture systems, filtration and sanitisation of water before intake.

2.20.1. White spot syndrome virus (WSSV)

WSSV continues to be one of the most serious disease problems faced by the shrimp farming industry worldwide. This virus was first reported in 1982 in _Fenneropenaeus japonicus_ cultured in northeastern Taiwan (Chou et al., 1995). Since then, WSSV has caused mortalities and consequent serious damage to the shrimp industry. Considering its virulent nature, wide host range, wide geographic distribution, high mortality, catastrophic economic losses, WSSV has become the single most–dangerous virus to the
penaeid shrimp farming industry. WSSV is known to affect most commercially important species of penaeid shrimp including *Penaeus monodon, P. chinensis, P. merguiensis, P. aztecs, P. stylirostris, P. vannamei, P. duorarum, P. setiferus F. japonicus* and *F. indicus* (Lightner, 1996a). Wild marine shrimp such as *P. semisulcatus, Metapenaeus dobsoni, M. monoceros, M. elegens, Heterocarpus sp., Aristeus sp., Parapanaeopsis stylifera, Solenocera indica, Squilla mantis* and fresh water prawn species *M. rosenbergii* have also been found to harbor this virus (Lo et al., 1996; Hossain et al., 2001a; Chakraborty et al., 2002). This virus has also been detected in many captured and cultured crustaceans and other arthropods including crabs (*Charybdis feriatus, C. annulata, C. lucifera, C. cruciata, Macrophthalmus sulcatus, Gelasimus marionis, Metapograpus messor, Scylla serrata, Sesarma oceanica, Matuta planipes, Helice tridens, Pseudograpsus intermedius*), pest prawn *Acetes* sp., small palaemonid prawn, larvae of Ephydridae insect and Artemia (Lo et al., 1996, Maeda et al., 1998, Otta et al., 1999, Chen et al., 2000, Hossain et al., 2001a, 2001b; Chakraborty et al., 2002).

Since WSSV can infect ovary, vertical transmission of the virus from brood stock to eggs has been demonstrated (Lo et al., 1997) and presence of WSSV in wild brood stock by a number of investigators (Lo et al., 1996, Otta et al., 1999). Lightly infected brood stock (nested PCR positive) may produce either infected or uninfected larvae. PCR is being commonly used in Asia to screen *P. monodon* larvae before stocking in ponds and the risk of crop loss has been reported to be high when larvae positive for WSSV by non-nested PCR are stocked. Simultaneous presence of WSSV, with other viruses such as monodon baculovirus (MBV) and hepatopancreatic parovirus (HPV) has been reported from India (Otta et al., 2003, Umesha et al., 2003).
2.20.2. *Monodon baculo virus* (MBV)

MBV is the first reported virus of *P. monodon* and the second virus of penaeid shrimp (Lightner and Redman, 1981). MBV has been identified and reported in *P. monodon*, *P. merguiensis*, *P. semisulcatus*, *P. kerathurus*, *P. vannamei*, *P. esculentus*, *P. penicillatus*, *F. indicus*, *Metapenaeus ensis* (Johnson and Lightner, 1988, Lightner, 1988, Chen et al., 1989a, Ramasamy et al., 1995, Vijayan et al., 1995, Karunasagar et al., 1998b). The occurrence of MBV with WSSV and HPV in hatchery reared *P. monodon* post larvae was reported for the first time in India by Manivannan et al., (2002). MBV is generally found in mixed infection with other pathogens including viruses (IHHNV, HPV, WSSV), bacteria (*Vibrio* spp, *Pseudomonas* spp), parasites (*Zoothamnium* spp, *Epistylis* spp.;(Anderson et al., 1987, Lightner et al., 1987, Chen et al., 1989a, Manohar et al., 1996, Karunasagar et al., 1998a, Umesha et al., 2003). Transmission of MBV occurs only horizontally through faecal oral route. MBV in the faecal matter of brood stock, infect eggs and larvae in hatcheries. The best way to eliminate MBV from hatchery is to identify carrier broodstock or to spawn females individually and discard contaminated batches of larvae (Flegel et al., 1995a).

2.20.3. *Hepatopancreatic parvo virus* (HPV)

The HPV was first reported by Lightner and Redman (1985) in postlarvae of *Penaeus chinensis*. In Thailand, HPV in the black tiger shrimp, *P. monodon*, was first reported in 1992 by Flegel and Sriurairatana (1993, 1994). Presence of HPV in *P. monodon* postlarvae in India was reported by Manivannan et al., (2002) and Umesha et al., (2003). This virus infects several penaeid species and is widely distributed in many parts of the world, including Asia, Africa, Australia and north and South America (Paynter et al., 1985, Colorni et al., 1987, Brock and Lightner, 1990, Fulks and Main, 1992, Lightner and Redman, 1992, Lightner,
1996a, Pantoja and Lightner, 2000). A number of cultured and wild penaeids have been reported as hosts for HPV (Flegel et al., 1992b, Bower et al., 1994). The transmission of HPV is believed to be both vertical and horizontal (Lightner and Redman, 1992).

2.20.4. Infectious hypodermal and hematopoietic necrosis virus (IHHNV)

IHHNV was first detected in juvenile *Penaeus stylirostris* from Hawaii in 1981 (Lightner et al., 1983). The virus has since been detected in a number of other penaeid species and from stocks around the world, including the Americas, Oceania and Asia (Lightner 1996a,b, Flegel, 1997b). IHHNV was detected earlier mainly by histological examination, but with the emergence of Polymerase chain reaction (PCR) this technique has been a handy tool in detection of this virus.

2.21. *Macrobrachium rosenbergii*

2.21.1. Habitat and biology of *M. rosenbergii*

Giant freshwater prawn species lives in tropical freshwater environments influenced by adjacent brackishwater areas. It is often found in extremely turbid conditions. Gravid females migrate downstream into estuaries, where eggs hatch as free-swimming larvae in brackishwater. After metamorphosis, post larvae assume a more benthic life style and begin to migrate upstream towards freshwater.

Larvae mostly consume zooplankton (mainly minute crustaceans), very small worms, and larval stages of other crustaceans. Postlarvae and adults are omnivorous, eating algae, aquatic plants, molluscs, aquatic insects, worms, and other crustaceans.

Males and females have different growth rates and males exhibit heterogenous individual growth (HIG); these are vitally important factors in grow-out management. Three distinct male morphotypes and a number of intermediary types exist. They are small male
(SM), orange claw males (OC), and blue claw males (BC). The normal male developmental pathway is SM → OC → BC. BC males have extremely long second pereiopods; those of OC males are golden coloured; SM have small, slim, almost translucent claws. The type and behaviour of the males affects the growth rates of other prawns. The transition from rapidly growing OC to the slowly growing BC morphotype follows a "leapfrog" growth pattern. An OC metamorphoses into a BC only after it has become larger than the largest BC in its vicinity. The presence of this new BC male then delays the transition of the next OC to the BC morphotype, causing it to attain a larger size following its metamorphosis. BC males dominate OC males, regardless of their size, and suppress the growth of SM (New et al., 2000).

2.21.2. History of the freshwater prawn culture

Although reared in captivity from time immemorial, modern farming of this species originated in the early 1960s when FAO expert Shao-Wen Ling, working in Malaysia, found that freshwater prawn (M. rosenbergii) larvae required brackish conditions for survival. This discovery led to larval rearing on an experimental basis. By 1972 the Hawaiian team led by Takuji Fujimura had developed mass rearing techniques for commercial-scale hatchery production of prawn post larvae (PL). This development spawned the first commercial farms in Hawaii and elsewhere. Both Thailand and Taiwan Province of China became pioneers in modern giant river prawn culture. The introduction of broodstock, initially from Hawaii and Thailand, into non-indigenous areas around the world began in the 1970s. Since then, giant river prawn culture has developed in every continent, particularly in Asia and the Americas (FAO, 2002). M. rosenbergii has been imported to many other tropical and subtropical areas of the world and is the species most favoured for farming purposes.
2.21.3. Broodstock management

When required for hatchery use, female broodstock are usually obtained from grow-out ponds but also sometimes from capture fisheries. Normally, "berried" (egg-carrying) females are only used once. Commercial farms in tropical regions do not normally maintain captive broodstock for breeding purposes but adults are over-wintered indoors in temperate regions in order to stock ponds with PL as early as possible in the short grow-out season. The typical male to female ratio in broodstock holding systems is 1-2 blue-clawed males or 2-3 orange-clawed males per 20 females, at a total stocking density of 1 prawn per 40 litres. Some seed (PL; juveniles) is obtained from the capture fishery where *M. rosenbergii* is indigenous, typically in the Indian sub-continent, but most is now hatchery reared (New *et al*., 2000).

2.21.4. Economic importance and market trends

The development of freshwater prawn farming was inhibited in the past by its longer hatchery phase and lower grow-out productivity compared to marine shrimp. These constraints are now balanced by a number of positive factors concerning its sustainability and the development of a distinct and expanding market niche for freshwater prawns. The culture of *Macrobrachium* spp. is less likely to have a detrimental impact because freshwater prawns cannot be reared at densities as high as those commonly used in marine shrimp farming. Productivity is generally lower, management is less labour intensive, and the potential for the abuse or waste of resources is minimal, and (unlike the inland culture of marine shrimp) the grow-out of *Macrobrachium* does not make agricultural land saline. Specific negative effects of *M. rosenbergii* culture on the environment have yet to be documented. It is particularly well-suited to small long-term family businesses, can be practised by relatively unskilled
fishing and rural people, generates products which may be consumed by all social classes, and is amenable to integration with crop production (FAO, 2006).

Farmed production is rapidly expanding in Asia. Production in India and Thailand expanded by more than 50 percent per year between 1999 and 2002; this trend is expected to continue. There is also potential for expansion in Bangladesh, a traditional exporter from its capture fisheries. In total, the output of *M. rosenbergii* from aquaculture expanded during the decade 1993-2002 from 17000 tonnes to 195000 tonnes, an APR of 31 percent/yr. Further global expansion is difficult to predict, since it depends mainly on the volume of consumer demand. However, even if a very modest expansion of 10 percent/year occurs, global farmed production of *M. rosenbergii* will have significantly exceeded 400000 tonnes by 2010 (FAO, 2007). Initially in India scampi was a subsidiary crop obtained from fish farms and over the years farmers have converted the system to a wholly scampi based one after realizing the profits earned from farming this species. Despite being third in world production status, the farming technology needs further improvement in order to improve productivity and profitability of the farms. Unlike fish or marine prawn culture where management is relatively easy, scampi culture is difficult owing to the complex social set up, behaviour and heterogeneous growth patterns between sexes.

Both domestic and international markets for the giant freshwater prawn exist and are expanding. Peeled, mostly wild-caught *M. rosenbergii* have long been exported globally, but farmed shell-on (and normally head-off) freshwater prawns are also a familiar sight in the supermarkets of Europe now. India, Bangladesh, Viet Nam and Thailand export a significant proportion of their wild-caught and farmed prawns. Freshwater prawns are a distinct product from marine shrimp, which have their own favourable culinary characteristics (FAO, 2007).