## INTRODUCTION

Lobsters are one of the most valuable and economically important crustaceans in India. Lobsters are widely distributed along the entire coast of India and major fisheries are located on the north-west, south-west, and south-east coasts (Radhakrishnan and Manisseri 2003). The north-west coast fishery is predominated with *Panulirus polyphagus* and Scyllarid *Thenus orientalis* (Chhapgar and Deshmukh 1971). In the south west *Panulirus homarus* (Linnaeus) and the deep sea water *Pureulus sewelli* are the foremost contributors to the fishery. Along the south east coast *Panulirus homarus* and *Panulirus ornatus* are the major contributors to the fishery (Radhakrishnan *et al.,* 2005). *P. homarus* (Decapopda, Palinuridae) is the most abundant and widespread crustaceans, distributed globally across tropical and sub-tropical regions of India especially in Cochin, Vizhinjam, Colachel, Muttom, and Tuticorin fishing harbors.

The fishery and biology of *P. homarus* studied during the last three decades were concentrating mainly on the movement, growth and fattening. But the population analysis, variations and biological reality of these animals are not yet explored in India. The population structure of a species is the result of a complex interaction between the life history characteristics (i.e. pelagic larval stages, homing behaviour), demographic parameters (i.e. recruitment, growth, reproduction, mortality) and genetic processes (i.e. drift, gene flow, selection, mutation). However, populations genetic data of these species provide the complete picture of the population structure—the most comprehensive definition of a *stock*.

Generally marine species analyses revealed less spatial variation in genetic structure than populations inhabiting freshwater environments. This is due to the large population size, high potential for dispersal and the perceived lack of barriers to dispersal (Graves 1998). In this regard *P. homarus* in Peninsular India has special interest, because peninsula has different geographical regions, its restricted movements and depleted population(Mohamed & George 1968, Radhakrishnan *et al.,* 2005). These reasons might restrict the gene flow in *P. homarus*. Genetic sub-structuring of a species is important knowledge for managing harvested species and can be used to predict whether a locally depleted population will be successfully repopulated by immigrants.

**Distribution and capture**

Geographical areas used in fisheries management do not typically coincide with the actual distribution areas of biological fish stocks simply because the stock may move seasonally between fixed management areas, or because separate fish stocks may occur in a single management area. This results in a mismatch between the spatial scale of management and biological reality (Daan 1991, Pawson and Jennings 1996). Uniform exploitation of a resource composed of numerous subpopulations/stocks, for example, can lead to the erosion of a section of that resource. In India, crustaceans have broad geographical distribution with more than 2934 species especially in four economic zones. The Indian southern peninsular area is fertile, having huge biodiversity facing heavy fishing pressure. About thousands of fishing vessels are operating in this area. The fisherman population maintains the seasonal catch and Total Allowable Catch (TAC) for individual species.

The spiny lobster *Panulirus homarus* (Linnaeus) is the most widely distributed among the three subspecies of *P. homarus homarus* and is found throughout the Indo-Pacific region with centers of high concentrations in East Africa and Indonesia (Berry, 1974, Pollock, 1993). The other two subspecies, *P. homarus megasculptus* and *P. homarus rubellus*, are restricted to the Arabian Sea and southeast coasts of Madagascar and Southern Africa, respectively. All the three subspecies inhabit shallow waters mostly between 1 and 5 m depth among rocks, often in the surf zone and sometimes in highly turbid estuarine areas (Holthuis, 1991). However, they respond to different environmental conditions that prevail in their respective areas (George, 1997).

**Capture**

The Indian peninsula has different geographical regions, which do not typically coincide with the actual distribution areas of lobster stocks (population). Major fishing grounds of *Panulirus homarus* in this area are Colachel and Muttom, where gill-net, trammel-net, and traps are used for catching lobsters. Annual landings gradually decreased from a peak of 301t in 1966 to 7.6t in 1996, with only 4t recorded in 2002. Among this 92% of the catch comprised of *P. homarus* (Radhakrishnan *et al.,* 2005). Increasing fishing effort, introduction of gill nets for fishing and exploitation of egg- bearing lobsters during the peak breeding season have been the major factors responsible for reduction in landings. *P. homarus* is an inshore species with restricted movements and is therefore highly vulnerable to fishing. Based on the current landing data and biological information on the mean size of *P. homarus*, it could be deduced that the stocks has been over exploited. Although the

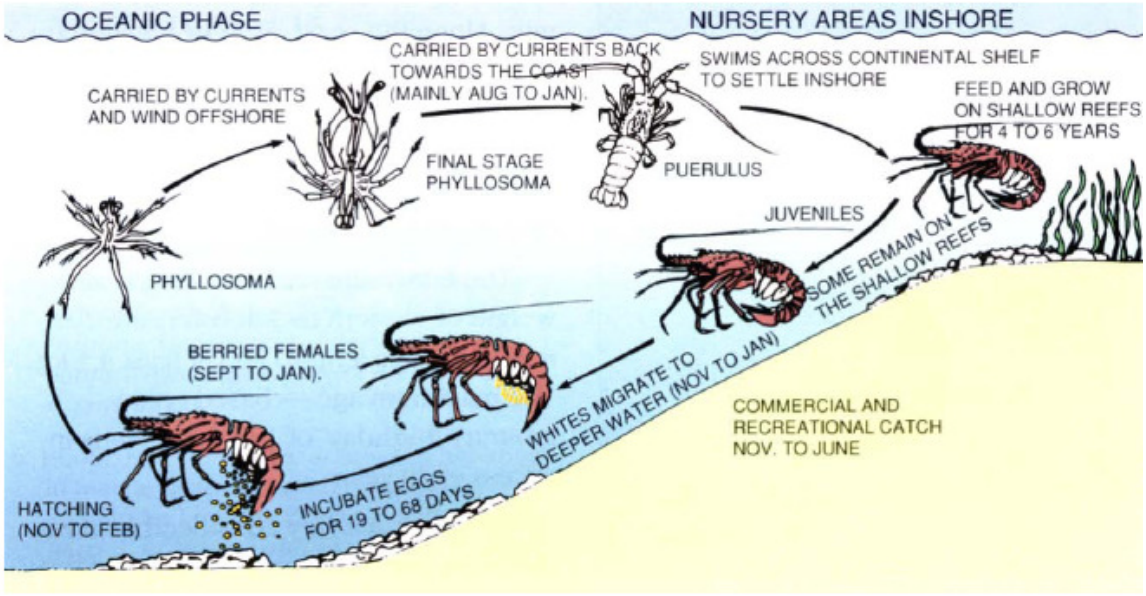
concept of stock identification is straightforward, the life history and ecology of a species can greatly affect the ability to actually identify genetic stocks in nature.

**Biology**

Lobsters are usually located at lower than mean low water neaps (sublittoral fringe) to depths of 150m. They are primarily nocturnal animals feeding on blue mussels, hermit crabs and polychaetes. Growth is by moult, which decreases in frequency during the juvenile stages until becoming an annual part of the mating, spawning and egg hatching cycle. Females can spawn annually or follow a bi-annual pattern. Reproduction takes place during summer and is linked with the moulting cycle. After extrusion, the eggs are held on the pleopods for approximately another year until hatching the following summer. Large females (>120 mm carapace length) have been shown to moult and then undergo two successive spawns before moulting again, suggesting the capacity for sperm storage. The first few post-hatching weeks are characterized by a pelagic phase usually lasting 14–20 days depending on the water temperature. During this period, larvae undergo four developmental stages until metamorphosis to stage IV (meta-larvae) when they settle to the seabed (**WoRMS**). In most areas lobsters do not mature before 5–8 years (depending on water temperature). Genetic data suggest that females in the wild mate with a single male. Results from tank experiments demonstrate that individual males can fertilize several females in the same season and this is likely to be the case in the wild. Thus the normal breeding system in the wild is likely to be polygynous. In the absence of exploitation the life span is probably in decade. Males reach

sexual maturity earlier than females. Lobsters are sedentary animals with home ranges varying from 2 to 10km (Holthuis, 1991).

**Fig: 3. Life cycle of *Panulirus homarus*.**




**Population genetics**

Population genetic structure of a species are shaped by both past and present ecological processes, such as recent paleoecological history (e.g., glaciations) and current pressures (e.g., over-exploitation, habitat degradation, introduction of invasive species). Taking into account the influence of present gene flow on the genetic structuring of the species is crucial in order to protect those populations with higher genetic diversity and greater ability to effectively be able to export individuals to other areas (Palumbi, 2004). Furthermore, recent paleoecological history should also be taken into account in order to protect regions containing higher diversity and distinct lineages (Fraser and Bernatchez,

2001). Neutral mtDNA markers can provide information about past events, while giving a picture of the overall gene flow between populations.

Population connectivity is mainly determined by the potential dispersal of the species, in a way that species with a restricted dispersive ability tend to present more genetically structured populations (Palumbi, 2004). Planktonic larval duration (PLD) is a key factor in shaping patterns of dispersal and degree of connectivity between populations of marine species (Shanks *et al.,* 2003). Thus, species with a longer PLD should present extensive gene-flow (panmixia) and, therefore, low or no population structuring. However, even though larvae can potentially disperse over long distances, population structuring can be found at lower levels than expected under the theoretical limit of larval dispersal (Taylor and Hellberg, 2003; Rocha *et al.,* 2008), given that larval behaviour coupled with oceanographic structures at different scales may be conducive to larval retention.

Achelata lobsters (spiny, slipper and coral lobsters) are decapod crustaceans characterized by the presence of the phyllosoma, a larval phase specially adapted for long time dispersal. Dispersive ability of phyllosoma larvae is among the highest found in crustaceans, with an estimated duration of up to 24 months in some species of the genus *Jasus* (Booth, 1994). Accordingly, no evidence of subdivision was found among *Jasus edwardsii* populations from Southern Australia or New Zealand (Ovenden *et al.,* 1997), among *Panulirus argus* populations from the Caribbean Sea (Silberman *et al.,* 1994), or *Palinurus gilchristi* populations from South Africa (Tolley *et al.,* 2005). On the contrary, RFLPs analysis of mtDNA showed a genetic subdivision in *Jasus verreauxi* populations from Southern Australia and New Zealand, probably due to a fall in larval survivorship while crossing the Tasman Sea. A clear differentiation was also found between P. argus

populations from the Caribbean Sea and Brazil (Diniz *et al.,* 2005), related to an oceanic barrier formed by the drop in salinity caused by the Amazon and Orinoco rivers. More recently, Gopal *et al.,* (2006) found shallow genetic partitioning for *Palinurus delagoae* along the South African coast, caused by the retention of some of the larvae in slow-moving anti-cyclonic eddies.

**Census vs. effective population size**

Census population size (*N*) refers to the total number of individuals in the population. Effective population size (*N*e) refers to that subset of individuals in the population that successfully contribute to the next generation. Factors that affect *N*e are discussed later. Populations with small *N*e values can suffer a reduction in genetic diversity, mainly as a consequence of faster genetic drift, which leads to loss or fixation of alleles (Nei *et al*., 1975).

In marine species (especially invertebrates and fish), high fecundity together with very large variance in reproductive success can lead to an *N*e several orders of magnitude smaller than *N* (Hedgecock 1994). Indeed the most recent estimates of *N*e in marine fish suggest sizes that are three to five orders of magnitude smaller than *N* (Turner *et al.,* 2002, Hauser *et al.,* 2002, Hutchinson *et al*., 2003). With such a small *Ne/N* ratio, even species with a very large *N* may suffer loss of genetic diversity due to fishing pressure. In short, millions and millions of individuals do not guarantee maintenance of genetic integrity.

**Estimating Ne**

Effective population size is notoriously difficult to estimate. The most reliable way is by temporal methods in which the same population is sampled through a time course in which it can/must be assumed that changes in allele frequencies are solely due to genetic drift. In other words, it must be assumed that there is no migration, no selection and no mutation. Even if these assumptions are accepted, the problem of sampling remains. Fortunately, historical DNA extracted from archived material such as scales (Nielsen *et al.,* 1999, Hauser et al. 2002, Ardren and Kapuscinski, 2003), fin clippings (Heath *et al.,* 1993) and otoliths (Hutchinson *et al.,* 2003, this study) solves this problem by providing the long term temporal sampling needed.

Classic methods for the calculation of Ne are based on changes in the standardised variance in allele frequency (F) (e.g. Nei and Tajima 1983, Waples 1989). The problem with these F-statistic approaches is that as they are summary statistics and therefore do not use all of the information in the data (Wang 2001) thereby reducing accuracy. Moreover, when applied to microsatellite data, these F-based methods have difficulty dealing with rare alleles, which are common in microsatellites and are sensitive indicator of genetic drift (Frankel and Soulé 1981). Furthermore the upper limits of 95% confidence interval often reach infinity (Luikart and Cornuet 1999), thus limiting the interest of these methods especially when Ne is large.

More recent methods for the estimation of Ne utilise maximum likelihood (ML) (e.g. Williamson and Slatkin 1999, Anderson *et al.,* 2000, Wang 2001). Although computationally intensive, these approaches which search for values that will maximise the

likelihood function, in our case the Ne that best fits the change in allelic frequencies in a model of only genetic drift are more accurate and less biased than F- based methods (Williamson and Slatkin 1999, Wang 2001) because they used all the information in the data.

**Factors affecting Ne**

Variance in reproductive success due to sweepstakes recruitment is considered to be the most important parameter for species with high fecundity and high initial mortality (type III survival curves) (Hedgecock 1994, Turner *et al.,* 2002), which are typical traits of marine fish. At equilibrium, the variance effective population size, as a function of N, the census size and of Vk, the variance in the number of offspring per parent, is Ne= (4N-4)/ (Vk+2) (Crow and Denniston 1988). With high levels of female fecundity (20,000-600,000 eggs·female-1) (Rijnsdorp 1991) offset by high levels of daily mortality for eggs (up to 20%) (Rijnsdorp and Jaworski 1990) and juveniles (up to 4%) (Van der Veer *et al.,* 1990), plaice falls into the type III category. Therefore, sweepstakes recruitment is probably the main factor reducing Ne for this species. However, additional factors can affect Ne, variance in reproductive success due to mating behaviour, uneven sex ratios and fluctuation of population size.

The importance of mating behaviour is often overlooked in marine fish mainly due to a lack of data. For plaice the commonly accepted model of free spawning in large, seasonal aggregations suggests little or no courting behaviour. In fact, very little is known about plaice mating behaviour in the wild due to the difficulties of observation. However, recent observations of captive flatfish indicate complex mating behaviours including the possibility

of female choice (Konstantinou and Shen 1995, Stoner *et al.,* 1999). If correct, this could increase the variance in reproductive success and would have a large effect on effective population size.

As there is sexual dimorphism in maturation and growth, as well as a difference in the natural mortality and vulnerability for fishing, the heavy fishing pressure in the North Sea has removed the larger adult specimens from the population more strongly in males than females. Consequently the sex ratio in plaice is slightly skewed towards females (Rijsndorp 1994), potentially leading to a reduction of Ne.

Fluctuations in population sizes are probably not very important in plaice compared to species like sardines or anchovies (Gaggiotti and Vetter 1999) as the long reproductive life span of plaice with overlapping generations creates a buffer against such fluctuations.

**Fisheries effects on genetic diversity and life history traits**

By reducing N overall and/or by disrupting reproductive behaviour, fishing pressure can reduce Ne, and, therefore, the genetic diversity. Such a loss of diversity can lead to a loss of adaptability. Empirical evidence is accumulating: Smith *et al.,* (1991) compared virgin and heavily exploited stocks of Orange Roughy (*Hoplostethus atlanticus*) in New Zealand and found an overall decrease in heterozygosity; a reduction in genetic diversity has also been reported in the New Zealand snapper (*Pagrus auratus*) (Hauser *et al.,* 2002) and in the North Sea cod (*Gadus morhua*) (Hutchinson *et al.,* 2003) both of which have a Ne/N ratio of 10-5. These two latter cases illustrate the fact that Ne in fish can be small enough to suffer a loss of genetic diversity under heavy fishing pressure.

It is usually considered that a Ne of 500 individuals is necessary to maintain genetic diversity for long term adaptability. With a Ne/N ratio of 10-5, this means that population with N less than 50 millions could already suffer loss of adaptability. How much such loss of genetic diversity contributes to the lack of recovery observed in most marine fisheries (Hutchings 2000) is still unclear.

**Molecular markers**

The dramatic development of molecular genetics since the first widespread use of allozymes in the 1970s, and currently exemplified by the Human Genome Project and other equally ambitious undertakings, has laid the groundwork for genomics. Popular genetic markers in the aquaculture community include mitochondrial DNA, Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Microsatellite, 16s rRNA gene, Single Nucleotide Polymorphism (SNP) and Expressed Sequence Tags (EST) markers. Future trends in DNA marker technology and means of exploiting research progress from the Human Genome Project and from model species such as zebra fish for the benefit of aquaculture genomics were reported (Rafalski, 2002; Vignal *et al*., 2002).

All organisms are subject to mutations as a result of normal cellular operations or interactions with the environment, leading to genetic variation (polymorphism). In conjunction with selection and genetic drift, there arises genetic variation within and among individuals, species, and higher order taxonomic groups. For this variation to be useful to

geneticists it must be (1) heritable and (2) discernable to the researcher, whether as a recognizable phenotypic variation or as a genetic mutation distinguishable through molecular techniques (Malau-Aduli *et al*., 2004).

Molecular marker technology can be applied to reveal these mutations. Polymorphisms could be caused by differences in nucleotide sequences at the priming sites (such as point mutations), or by structural rearrangements within the amplified sequence (e.g., insertions, deletions, inversions) (Welsh and McClelland, 1990). Point mutations are more difficult to detect because they do not cause changes in fragment sizes.

**Random amplified polymorphic DNA (RAPD)**

RAPD procedures were first developed in 1990 (Welsh and McClelland, 1990; Williams *et al*., 1990) using PCR to randomly amplify anonymous segments of nuclear DNA with an identical pair of primers 8–10 bps in length. Because the primers are short and relatively low annealing temperatures (36–40ºC) are used, the likelihood of amplifying multiple products is great, with each product represents 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. Genetic variation 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 potential power is relatively high for detection of polymorphism; typically, 5–20 bands can be produced using a given primer pair, and multiple sets of random primers can be used to scan the entire genome for differential RAPD bands (Liu, 2004). 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).

RAPDs have all the advantages of a PCR-based marker, with the added benefit that the primers are commercially available and do not require prior knowledge of the target DNA sequence or gene organization. RAPD makers have been used for species identification in fishes (Partis and Wells, 1996) and mollusks (Klinbunga *et al*., 2000; Crossland *et al*., 1993), analysis of population structure in black tiger shrimp (Tassanakajon *et al*., 1997) and marine algae (Van Oppen *et al*., 1999), analysis of genetic impact of environmental stressors (Bagley *et al*., 1998), and analysis of genetic diversity (Wolfus *et al*., 1997).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 (Waldman, 1999).

Genetic and environmental factors are considered to play important roles in determining larval quality of penaid prawn*s* and the poor performance of pond-reared spawners of the giant tiger prawn, *Penaeus monodon* (Benzie *et al.,* 1997). Environmental factors such as water quality or the lack of key nutritional elements (Primavera *et al.,* 1985) and genetic effects such as inbreeding depression have been responsible for the poor performance of pond-reared animals (Shordoni *et al.,* 1987). Although little progress have been made in understanding the nature and degree of these influences on larval quality (McVey, 1993).

Temperature, day length, light quantity and quality, water qualities such as salinity, pH, dissolved oxygen concentration, nitrate levels and heavy metal concentration have been demonstrated to have an influence on egg and larval quality (Yuan *et al.,* 1992).

RAPD 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 has also been used for phylogenetic studies for species and Skibinski, 1994; Borowsky et al., 1995; Sultmann *et al.,* 1995; Corely- Smith *et al.,* 1996) and for gene mapping studies in fish (Postlethwait *et al.,* 1994; Kaizianis *et al.,* 1996).

Standardization and internal control of this technique is necessary to obtain reproducible profiles by RAPD (Ellsworth *et al.,* 1993). The genetic polymorphism of six populations *T. flavida* was assessed by optimized RAPD technique (Jorge Fraga *et al.,* 2005). Grace *et al.,* (2000) studied the extent of genetic variation within and between populations of *Ampiura filiformis* with different life history strategies using RAPD techniques. The fact that arbitrarily primers are potentially capable of amplifying numerous loci in the genome and that the produced DNA fragments have a taxon-specific nature, makes the RAPD technique particularly attractive for the analysis of genetic distance and phylogeny reconstruction (Borowsky *et al.,* 1995).

**Microsatellites**

Microsatellites are short DNA sequences (2-5 base pairs), tandemly repeated and scattered throughout the genome of most eukaryotes. They are nuclear, co-dominant, single-locus, and highly polymorphic. For example, 10-20 alleles/ locus are common although hypervariable loci with upto 100 alleles/locus are also possible. In the present study the number of alleles ranged from 5 to 40/locus. Length variation in repeat number allows for easy genotyping, especially with automated methods that permit direct sizing of the DNA fragments (alleles).

In short, microsatellite loci are the current marker of choice for population genetic studies requiring allele frequency data, such as studies of population differentiation and the mating system (Jarne and Lagoda 1996). Due to the high variability and abundance of microsatellites throughout the genome, their use as markers for DNA fingerprinting has been found to be a powerful tool for population genetic studies (Reilly and Wright, 1995; Wright and Bentzen, 1995) and pedigree reconstruction, especially for communally reared populations (Herbinger *et al.,* 1995). In order to assess the utility of microsatellite DNA markers for detecting changes of genetic diversity in hatchery strains and for estimating their genetic relationships in pacific abalone by using six primers. (Qi Li *et al.,* 2004). Microsatellite genotyping has proved to be an efficient tool for examining genetic structure (Wright and Bentzen, 1995) and pedigree tracing of hatchery populations, from various aquatic organisms (Goldstein and Scholterer, 1999). Microsatellites are genetic marker that have use in distinguishing population (Balloux and Lougon- Moulin, 2003), and in monitoring pedigree and genetic diversity in hatchery–reared organisms, such ass fish

shellfish, the objective being to determine the impact of stock enhancement activities on wild populations (Perez- Enriquez and Taniguichi, 1999; Perer-Enriquez *et al.,* 2001; Boudry *et al.,* 2002).

Despite all of their advantages, microsatellites are not without problems. Loci must be developed on a species-specific basis (although cross-species amplification is sometimes possible) which require considerable time and expense. Development of 6-10 suitably polymorphic loci takes from 3-9 months in fish depending upon specific optimisation problems encountered. A more serious problem with microsatellite loci is their mutation model and their rapid mutation rate. Earlier generation co-dominant markers (allozymes) were assumed to evolve relatively.

Slowly and under an infinite alleles model (IAM). In contrast, microsatellite loci have mutation rates estimated between $10^{-3}$ and $10^{-5}$/generation combined with a step-wise mutation model (SMM) in which the repeat length may expand or contract through replication slippage (Levinson and Gutman 1987). Fast mutation combined with forward and backward mutation may lead to homoplasy and saturation of a locus. In order to account for this SMM mutation model, Slatkin (1995) developed RST for estimating population differentiation. However, as more data became available, it appears that most microsatellites do not follow a strict SMM (Webster *et al.,* 2002) and other models, such as a Two Phase Model (Dirienzo *et al.,* 1994), have been proposed. It is now recognised that for population

differentiation studies the traditional FST (Wright 1969) is still preferred over RST (Gaggiotti *et al.,* 1999).