SUMMAARY

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Analysis of genetic structure is especially important for species with ecological and commercial value. Molecular analyses are very important in order to conduct an adequate monitoring of captive stocks and to establish profitable genetic improvement programs. The importance of genetic evaluation in Marine Organisms has been increased significantly to maintain genetic diversity and improve the fisheries management.

Panulirus homarus (Linnaeus) is the prime contributor to the fishery south west and south east part of the peninsular India. The genetic diversity of *Panulirus homarus* from different geographic regions were examined by using popular genetic markers RAPD, Microsatellites and 18s rRNA gene. In total, 250 individuals were sampled (50/location) from five different spots along the coast of Indian Peninsula such as Cochin, Vizhinjam, Muttom, Chinnamuttom and Tuticorin. The sampling site represents a diverse distribution of its habitats in India.

The whole study is divided into 3 chapters. **Chapter1** gives the details of the investigation of genetic polymorphisms of populations by using RAPD markers. It is archived in historical DNA extracted from the tissue samples. The development of new microsatellite loci for *P. homorus* is described and evidence for population differentiation throughout the Indian peninsula, including east and west coast, was explored using the microsatellite loci in **chapter 2**. In this analyses, adult and juvenile

(=young-of-the-year) samples were taken from 5 locations. In both analyses

hypothesised connections among feeding, spawning and nursery grounds were determined and differences in the properties and resolving power of the markers compared. This result led us to investigate the possibility of relatedness and, ultimately, inbreeding a condition not usually considered in species with large census populations. **Chapter 3** reports on lobsters (*P. homorus*) 18s rRNA gene polymorphisms from different geographic regions of Indian peninsula.

The frozen tissue samples of *P. homarus* were used for DNA extraction (phenol or chloroform method). The purity of isolated DNA was estimated using UV spectrophotometer by ratio of absorbance reading between 260 and 280 nm. The quality of DNA was tartan visually on 1% agarose gel.

RAPD authentication

Genetic variations of *Panulirus homarus* from different locations were examined using randomly amplified polymorphic DNA (RAPD). Among the Eight different decamer primers were tested in *P. homarus* populations, one primer (5'- AGCATTAGGG -3' GC: 50%) was generated reproducible bands; hence they was chosen for further analysis. The banding patterns produced showed the variations in each population. The primer produced 5 to 7 RAPD fragments in each population. In general the number and size of bands generated, strictly depend upon the primer used and the source of the template DNA. The primer produced least bands in Vizhinjam populations (5 bands) and six RAPD fragments in Chinnamuttom populations. Seven RAPD fragments were produced Cochin, Muttom and

Tutucorin populations. In Vizhinjam population two DNA fragments were not produced. This might be due to the gene mutation.

Among the five populations highest genetic distance was found between the populations Cochin and Muttom (0.93103). Lowest genetic distance was found between the populations Muttom and Tuticorin (0.83636). Based on Phylogenic tree analysis three clusters were formed.

The results of the present study clearly point out that each geographical sites has there influences on the gene pool of *P. homarus.* It was found that there is distinct genetic variation among the DNA from different populations. The populations from Cochin, and Vizhinjam, gene pool grouped together as a single cluster, and Muttom and Tuticorin grouped together as a cluster whereas the population from Chinnamuttam had distinct genetic cluster and is unique in fingerprints. This variability can be accounted due to the ecological differences of the Arabian Sea and Bay of Bengal. Populational genetic differentiation can be driven by ecological, evolutionary, geographical barriers, overexploitation and historical factors.

Microsatellites

Microsatellite marker analysis of the five populations from different geographic regions reveals the prevailing genetic differentiation between the populations. Among the five primers tested, two primers were produced amplified fragments in all populations. The primer PH2 produced 4 to 7 DNA fragments. Least bands are produced in Chinnamuttom, Cochin and Tuticorin. Highest numbers of fragments were produced in Vizhinjam

populations. Allele polymorphic bands were produced in the Tuticorin and Vizhinjam populations. Primer (PH2) Microsatellite distance matrix were generated by using Jaccard NJ Method. The genetic distance (0.48684) was found in Tuticorin population with Muttom and Chinnamuttom populations. The lowest genetic distance was found between Muttom and Cochin populations.

The primer PH5 produced 10 to 12 DNA fragments. Allele polymorphic bands were produced in all populations. Primer (PH5) Microsatellite distance matrix were generated by using Jaccard NJ Method. The genetic distance (0.54829) was found between Cochin and Muttom populations. Lowest genetic distance was found between Muttom and Tutucorin populations (0.51266). Phylogenic tree constructed using Neighbour joining algorithm has three clusters. First cluster contains Muttom populations, second cluster contains Tuticorin populations and third cluster contains Vizhinjam, Chinnamuttom and Cochin populations. These results demonstrated that the variability in populations due to the ecological differences of the Arabian Sea and Bay of Bengal and life historical factors.

18S rRNA

The 18s rRNA gene was amplified and sequenced by using primer Forward and reverse primer. Multiple Sequence alignment and Phylogenetic distance tree constructed using UPGMA method. 18S gene sequences data of five populations has more or less same alignment length with 1797 nucleotide bases. Also, there was a significant difference in the percentage composition of nucleotide contents (adenine, thymine, cytosine, and guanine) between the collection sites. A significant difference was also found in relative frequencies

of nucleotide substitutions (adenine-guanine, cytosine-thymine, adenine-cytosine, adenine-thymine, cytosine- guanine, and guanine-thymine).

On the basis of Phylogenic tree highest genetic distance was found in Tuticorin population (0.205589) and less genetic distance found in Vizhinjam population (0.00583658). Phylogenic tree produced mainly two clusters. First cluster contains Tuticorin populations and the second cluster contains remaining four populations. The results of multiple sequence alignment of 18S gene sequence in five different collection spots showed that the base pairs varied in all populations. It revealed that these variations are caused by the environmental conditions, geographical barriers, overexploitation and life history.