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RESEARCH ARTICLE

DETERMINATION OF GENETIC DIVERSITY IN *PORTUNUS PELAGICUS* (LINNAEUS, 1758) COLLECTED FROM GULF OF MANNAR

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Abstract

In the present study, the marine crab *Portunuspelagicus* was screened with 17 primers and made to score polymorphism. The main emphasis of the present study was to assess the genetic diversity at intra specific level among the 3 accessions of *P. pelagicus* species of Gulf of Mannarusing RAPD markers. RAPD analysis shows that there is a high level of polymorphism among different accessions. From this study, it was understood that each location varied with respect to environmental factors and genetic parameters. The OPB-18, OPB-19, OPC-07 and OPN-06 primers produced distinct, highly reproducible amplification profile for all the screened samples. In the present study, amplification bands ranged between 250 and 663 bp. Maximum numbers of bands were produced by OPN-06 and least by OPB-19. Moderate to high genetic diversity was observed in all geographic samples of *P. pelagicus* from RAPD analysis. RAPD analysis from three different geographical regions shows clear polymorphic patterns. Thoothukudi and Rameshwaram populations appear in one cluster, while the Kanyakumari populations formed the other cluster indicating a genetic variability and diversity in samples collected from different places.

Introduction:-

Genetic diversity refers to the variation of genes within species. This constitutes distinct population of the same species or genetic variation within population or varieties within a species. Genetic diversity resides in the variation in the sequences of four base pairs, such as components of nucleic acids which constitute the genetic code. Other kinds of genetic diversity can be identified at various levels of organizations, including the amount of DNA present, chromosome structure and number. New genetic variations arise in individuals by gene and chromosomal mutations, and in organisms with sexual reproductions, these variations are spread through the population by recombination [1]. Genetic diversity deals with the genetic characteristics of the organisms that play a major role in the survival and adaptability of the species [2].

The ability of organisms to challenge the adaptability to their changing environment leads to its survival and causes rise in genetic variation [3]. Genetic variations can be determined based on morphological, molecular and biochemical types of information [4,5&6]. Genetic variation in fishes has proved to be valuable in fisheries and aquaculture management for identification of stocks, and in selective breeding programmes. Individuals with larger genetic variability have higher developmental stability, growth rates, fecundity, viability, and resistance to

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environmental stress and diseases [7]. A species may undergo micro-evolutionary processes and differentiate into genetically discrete sub-populations or stocks in the course of time, if geographically and reproductively isolated.

The combined effects of deterministic factors such as habitat loss, pollution, introduction of exotic species, over exploitation and stochastic factors such as catastrophic effects, environmental changes, demographic effects, genetic factors are being considered to be major threats in biological extinction [8]. In addition the deleterious effects of inbreeding and affirmed genetic factors had an important role in causing species extinction. Moreover the accumulation of mutations in the genome has the capacity to determine the fitness of the organisms [9].

Large numbers of highly informative DNA markers have been developed for the identification of genetic polymorphism. In the last decade, the random amplified polymorphic DNA (RAPD) technique based on the polymerase chain reaction (PCR) has been one of the most commonly used molecular techniques to develop DNA markers. RAPD markers are amplification products of anonymous DNA sequences using single, short and arbitrary oligonucleotide primers, and thus do not require prior knowledge of a DNA sequence [10]. Molecular markers have greater advantages. They demonstrate genetic differences in depth level without interferences from environmental factors, offering quick results giving a detailed review on genetic diversity [11,6]. Segments of chromosomes which don't essentially encode any traits are molecular markers, not affected by the environment but inherited in a Mendelian fashion. Some segments of the chromosome get modified sooner than others (i.e. non-coding vs. coding DNA). As a consequence it is suggested to utilize slow changing markers for less related individuals (different species) and fast changing markers for closely related individuals. RAPD markers have found a wide range of applications in gene mapping, population genetics, molecular evolutionary genetics etc. This is due to the speed, cost and efficiency of the RAPD technique to generate large numbers of markers in a short period compared with previous method.

Crustacea is a class of organisms under the phylum arthropoda, which includes crabs, lobsters, barnacles and shrimps [12]. All arthropods have exoskeletons and jointed appendages. The class crustacea includes arthropods that breathe using gills or branchiae, and possess two pairs of antennae. Most marine arthropods are crustaceans. Crabs, among numerous other invertebrates are considered as an essential shell fishery product [13]. The blue swimming crab, *P. pelagicus*also known as sand crab is one of the most important commercial marine species and recreational fisheries. It is distributed from the intertidal zone to approximately 50 m depth. The blue swimming crab exhibits moderately long planktonic larval stages (26-45 days) and high mobility during the crab phase [14]. Therefore, high gene flow levels are expected in this species [15].

In India, these crabs spread almost throughout the coastal island. Several key indicators show that *P. pelagicus* population is in crisis due to overexploitation. In the present study, genetic diversity of the species of crab *P. pelagicus* from three different geographical locations was carried out using RAPD PCR, a nuclear type II marker.

Materials and Methods:-

The DNA extracted for DNA barcoding studies were used as the template in RAPD PCR.

Random Amplification of Polymorphic DNA (RAPD) :

The principle is that, a single, short oligonucleotide primer, which binds to many different loci, is used to amplify random sequences from a complex DNA template. This means that the amplified fragment generated by PCR depends on the length and size of both the primer and the target genome. The assumption is made that a given DNA sequence (complementary to that of the primer) will occur in the genome, on opposite DNA strands, in opposite orientation within a distance that is readily amplifiable by PCR. These amplified products (of up to 3.0 kb) are usually separated on agarose gels (1.5-2.0%) and visualized by Ethidium bromide staining. The standard RAPD utilizes short synthetic oligonucleotides (10 bases long) of random sequences as primers to amplify nanogram amounts of total genomic DNA under low annealing temperatures by PCR. Primers are commercially available from various sources (e.g. Operon Technologies Inc., California; Biosciences, Bangalore; Eurofinns, Bangalore; GCC Biotech, Kolkata). In RAPD analysis, the target sequence(s) (to be amplified) is unknown. A primer is designed with an arbitrary sequence. In order for PCR to occur: 1) the primers must anneal in a particular orientation (such that they point towards each other and, 2) they must anneal within a reasonable distance of one another. Figure 2 depicts a RAPD reaction, a large fragment of DNA (genome A) is used as the template in a PCR reaction containing many copies of a single arbitrary primer.

PCR was carried out in an Eppendorf Master Cycler Personnel Thermo cycler using the following program.

Primers for study: OPA19, OPC7, OPB18 and OPN6:
OPB-18 CCACAGCAGT
OPB-19 ACCCCCGAAG
OPC-07 GTCCCGACGA
OPN-06 GAGACGCACA
Reaction set up for RAPD

Components	Stock concentration	Final concentration	Volume for 20µl Setup
MilliQwater			11.53 µl
dNTP mix	2 mM	0.2 mM	2 µl
$MgCl_2$	25 mM	0.375 mM	0.3 µl
Taq buffer	10X	1X	2 µl
RAPD PRIMERs	3 µm	0.4 µm	2.67 µl
DNA Template	100 ng/µl	100 ng	1 µl
Taq DNA polymerase	5 U/µl	2.5 U	0.5 µl

PCR Reaction conditions:

Initial denaturation : 94°C – 3 min
Denaturation : 94°C – 45 sec
Annealing : 37°C – 1 min
Extension : 72°C – 1 min 20 sec
Final extension : 72°C – 7 min
Hold : 4°C
Total number of cycles 40
The samples were run on a PAGE Gel

Polyacrylamide Gel Electrophoresis Technique:
PAGE (Polyacrylamide Gel Electrophoresis), is an analytical method used to separate components of a protein mixture based on their size. The technique is based upon the principle that a charged molecule will migrate in an electric field towards an electrode with opposite sign. The general electrophoresis techniques cannot be used to determine the molecular weight of biological molecules because the mobility of a substance in the gel depends on both charge and size. To overcome this, the biological samples need to be treated so that they acquire uniform charge, then the Electrophoretic mobility depends primarily on size. For this different protein molecules with different shapes and sizes, need to be denatured (done with the aid of SDS) so that the proteins lose their secondary, tertiary or quaternary structure. The proteins being covered by SDS are negatively charged and when loaded onto a gel and placed in an electric field, it will migrate towards the anode (positively charged electrode) are separated by a molecular sieving effect based on size. After the visualization by a staining (protein-specific) technique, the size of a protein can be calculated by comparing its migration distance with that of a known molecular weight ladder (marker).

The gel used is divided into an upper "stacking" gel of low percentage (with large pore size) and low pH (6.8), where the protein bands get squeezed down as a thin layer migrating toward the anode and a resolving gel (pH 8.8) with smaller pores. Cl^- is the only mobile anion present in both gels. When electrophoresis begins, glycine present in the electrophoresis buffer, enters the stacking gel, where the equilibrium favors zwitterionic form with zero net charge. The glycine front moves through the stacking gel slowly, lagging behind the strongly charged, Cl^- ions. Since these two current carrying species separate, a region of low conductivity, with high voltage drop, is formed between them. This zone sweeps the proteins through the large pores of the stacking gel, and depositing it at the top of the resolving gel as a narrow band.

Non-denaturing 10% polyacrylamide gels were prepared according to standard protocols. Fragment separation was done in a vertical electrophoresis unit.

Page gel:

1. The glass plate is assembled on a clean surface. The longer glass plate (the one with spacer) is laid down first, then placed the shorter glass plate is placed on top of it.
2. They are embedded into the casting frame and clamped properly. It is made sure that the bottom ends of the glass plates are properly aligned.
3. It is then placed on the casting stand.
4. The separating gel solution is prepared by combining all reagents.
5. APS and TEMED are added to the monomer solution (just before pouring) and mixed well by swirling gently. The solution is poured till the mark. (It is ok if you introduce air bubbles, a layer of isopropanol or distilled water is added on top of the gel so as to level the poured gel).
6. The gel is allowed to polymerize for 20-30 min.
7. The stacking gel is prepared by mixing all reagents except APS and TEMED. The isopropanol is drained with strips of filter paper.
8. APS and TEMED are added to the monomer solution (just before pouring) and mix well by swirling gently. (Make sure you keep the comb ready by the side).
9. A comb is placed in the stacking gel sandwich. It is allowed to polymerize for 10 min.
10. When the plates are secured in the gel apparatus, they are placed in the cassette and then locked.
11. The inner chamber of the tank is filled with buffer.
12. The comb is carefully removed (without breaking the well). [Now the gel is ready to load the samples]
13. The loading tip is inserted to a few mm from the well bottom and delivered the samples into the well.
14. The power supply is attached by putting the lid (Make sure that the connection is in correct way i.e., black - black and red - red). The voltage is set up to 180 V and run for 1 hour.
15. After running, power supply is switched off and the gel plates taken out. The gel is carefully removed and placed in the staining solution.

Silver staining:

Highly sensitive detection of nucleic acids in the nanogram range has been achieved by the specific chemical reduction of silver ions. The methods for silver-staining nucleic acids employ either a histologically derived procedure that uses ammoniacal solutions of silver, or a photochemically derived reaction in which silver binds to nucleic acid bases and is then selectively reduced by chemical agents or light. These silver staining protocols can be as sensitive as radioisotopic methods. However, they are complex and time consuming and require the preparation and handling of several solutions. In an attempt to simplify the routine use of silver stains to detect nucleic acids,has optimized the photochemically derived silver stain originally introduced for protein staining and later applied to nucleic acids which uses formaldehyde to selectively reduce silver ions to metallic silver under alkaline conditions.

Step	Time	Reagent
Fix	30 min	10% acetic acid (v/v)
RINSE	3 × 2 min	Distilled water
IMPREGNATE	20 – 30 min	1 % $AgNO_3$ (g/l) + 100 µl 37% formaldehydes per 100 ml of Distilled water
RINSE	3 × 2 min	Distilled water
DEVELOP	2 – 5 min	2.5% Na_2CO_3 + 100 µl 37% formaldehydes per 100 ml + $Na_2S_2O_3.5H_2O$ (2 mg/liter)
STOP	10 min	2% glycine (g/v) + 0.5% EDTA
PRESERVE		5% Glycerol (v/v)

The gel is viewed in White light Illuminator

Population genetic analysis:

For the analysis of the RAPD-PCR data set, the gels were examined and all the amplified loci were treated as diploid dominant markers. The amplified bands were then visually scored as binary variables where 1 represented band present and 0 represented band absent. Each marker band was assumed to be representing a distinct locus. The presence of a band represents the dominant genotype (Homozygote and heterozygote) and absence of band represents the recessive homozygote genotype. A Dendrogram, summarizing the genetic relationships among all populations was built using the SPSS Software.

Results:-

Screening of primers:

In this study preliminary screening of 17 primers was made to score polymorphism. However, 13 Operon series primers did not show consistency in amplification of bands in all the samples. The OPB-18, OPB-19, OPC-07 and OPN-06 primers produced distinct, highly reproducible amplification profile for all the screened samples. These 4 primers were selected for RAPD analysis of *Portunus* species. The size of the amplified bands varied with different primers and with DNA sample of different provenances.

Four primers (OPB-18, OPB-19, OPC-07 and OPN-06) provided 27 amplification bands, ranging between 250 and 663 bp. Maximum numbers of bands were produced by OPN-06 (14) and least by OPB-19 (2) . Moderate to high genetic diversity was observed in all geographic samples of *P. pelagicus* from RAPD analysis. The data of the total number of bands (TNB), number of polymorphic bands (NPB), percentage of polymorphic bands (%PB), amplified fragment size (AFS) obtained for each primer was calculated. The percentage of polymorphic bands in each primer across overall samples was 63%. Quite a number of polymorphic bands found in the RAPD analysis suggested moderate to high genetic diversity of *P. pelagicus* along the Gulf of Mannar coast.

CODE	Sequence 5' to 3'	No of Amplified products
OPN18	CCACAGCAGT	5
OPN19	ACCCCCGAAG	2
OPC7	GTCCCGACGA	6
OPN6	GAGACGCACA	14

Figure 1-4 represent the comparison of the RAPD patterns of the crab representing various coastline of *P. pelagicus* obtained from primers OPB-18, OPB-19, OPC-07, and OPN-06.

Dendrogram Analysis:

SPSS Dendrogram based on all 4 primers indicated *P. pelagicus*collected from Station-I (Kanyakumari-CinnaMuttam) coast has the most distinct population.

The main emphasis of the present study was to assess the genetic diversity at intra specific level among the 3 accessions of *P. pelagicus* species of Indian subcontinent using RAPD markers. RAPD analysis shows that there is a high level of polymorphism among different accessions. From this study, it was understood that each location varied with respect to environmental factors and genetic parameters.

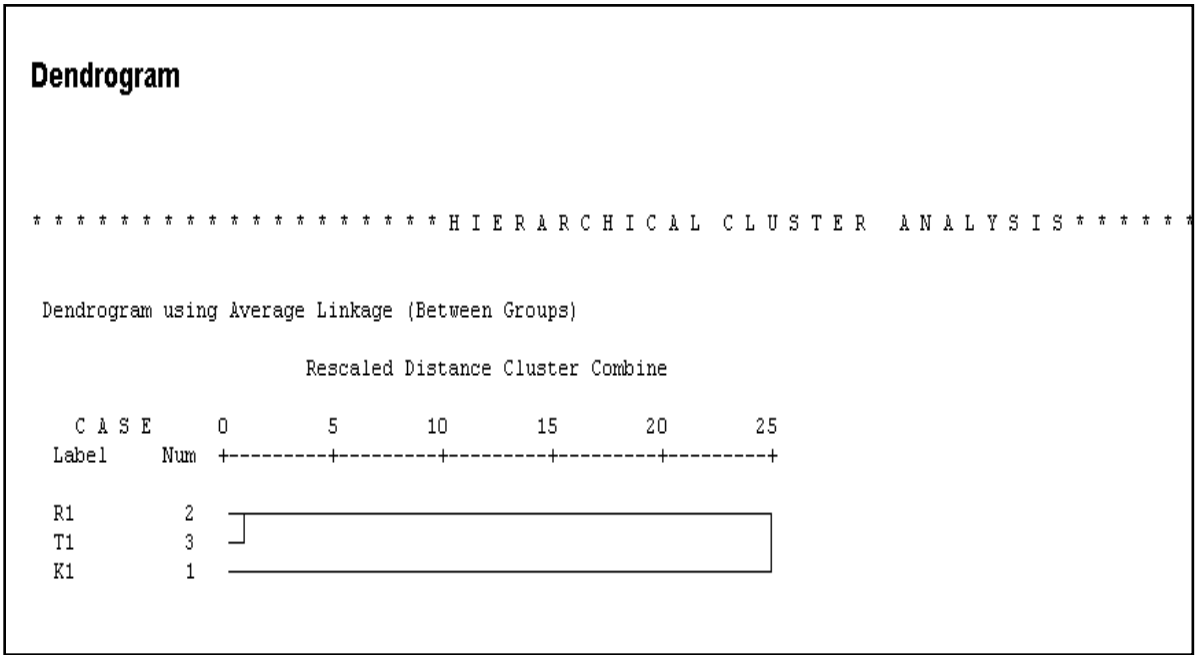
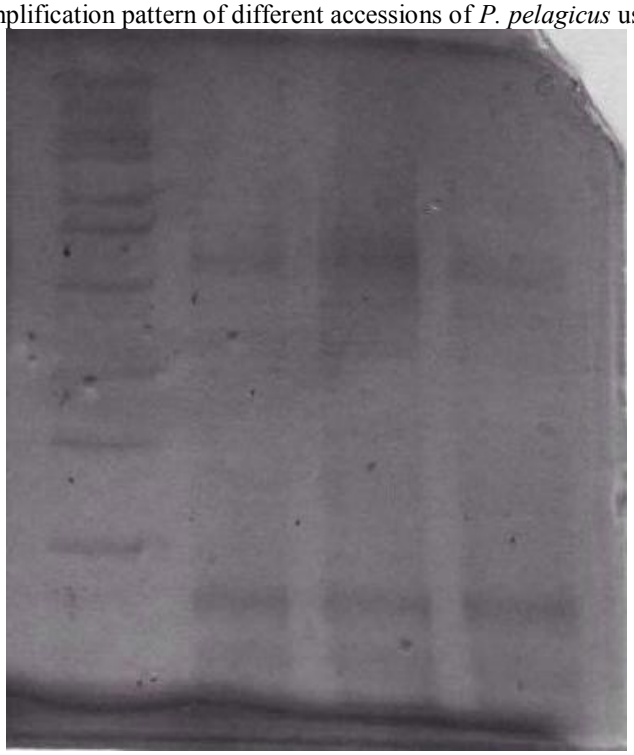
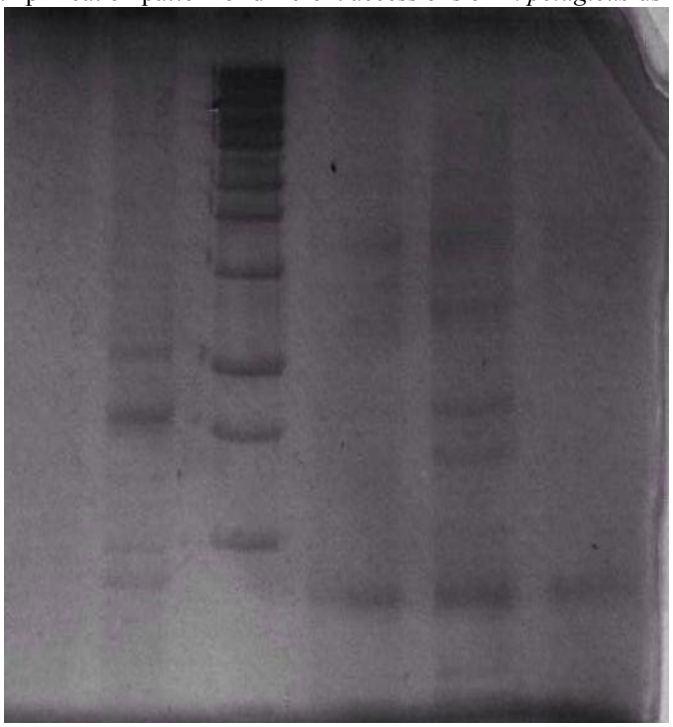


Figure 1:- RAPD amplification pattern of different accessions of *P. pelagicus* using primer OPB-18.



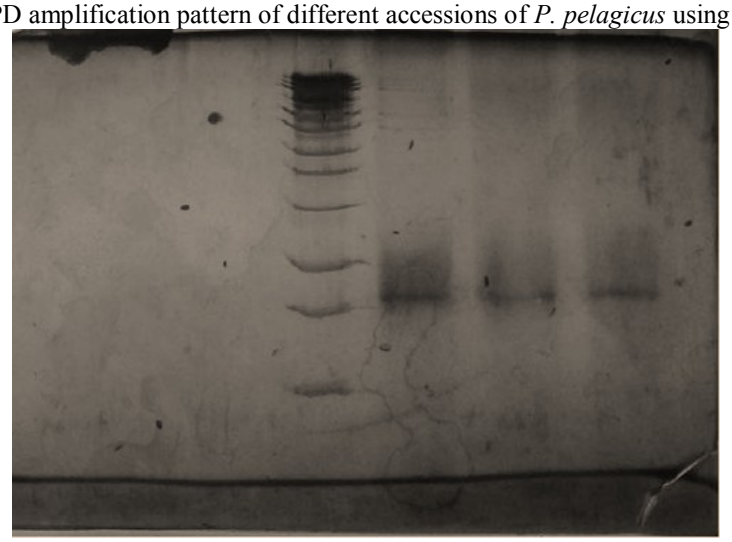
Lane 1 Lane 2 Lane 3 Lane 4
Lane 1: 1 Kb Ladder; Lane 2: Station - I (K); Lane 3: Station- III (R);
Lane 4: Station- II (T)

Figure 2:- RAPD amplification pattern of different accessions of *P. pelagicus* using primer OPB-19.



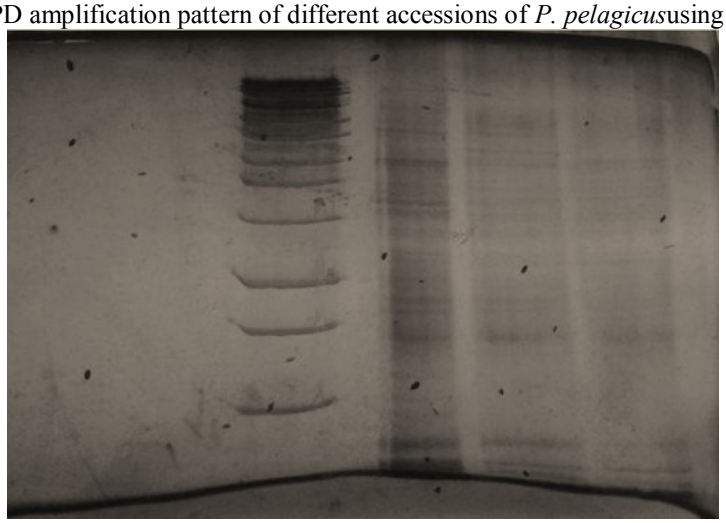
Lane 2 Lane 3 Lane 4 Lane 5
Lane 2: 1 Kb Ladder, Lane 3: Station - I (K), Lane 4: Station- III (R),
Lane 5: Station- II (T)

Figure 3:- RAPD amplification pattern of different accessions of *P. pelagicus* using primer OPC-7.



Lane 1 Lane 2 Lane 3 Lane 4
Lane 1: 1 Kb Ladder; Lane 2: Station - I (K); Lane 3: Station- III (R);
Lane 4: Station- II (T)

Figure 4:- RAPD amplification pattern of different accessions of *P. pelagicus*using primer OPN-6.



Lane 1 Lane 2 Lane 3 Lane 4
Lane 1: 1 Kb Ladder; Lane 2: Station - I (K); Lane 3: Station- III (R);
Lane 4: Station- II (T)

Table 1:- RAPD Gel scoring pattern of different accessions of *P. pelagicus* using primer OPB-18.

Band size	Station I	Station II	Station III
250	0	0	0
400	1	1	1
500	0	0	0
750	0	0	0
850	0	0	0
1000	0	0	0
1400	0	0	0
1500	0	0	0
1750	1	1	1
2000	0	0	0

3000	0	0	0
4000	0	0	0

Table 2:- RAPD Gel scoring pattern of different accessions of *P. pelagicus* using primer OPB-19.

Band size	Station I	Station II	Station III
250	0	0	0
400	1	1	1
500	0	0	0
750	0	0	1
850	0	0	1
1000	0	0	0
1400	0	0	1
1500	0	0	0
1750	1	0	1
2000	0	0	0
3000	0	0	0
4000	0	0	0

Table 3:- RAPD Gel scoring pattern of different accessions of *P. pelagicus* using primer OPC-7.

Band size	Station I	Station II	Station III
250	0	0	0
400	0	0	0
500	0	0	0
750	0	0	0
850	1	1	1
1000	0	0	0
1400	0	0	1
1500	0	0	0
1750	1	0	1
2000	0	0	0
3000	1	0	0
4000	1	0	0

Table 4:- RAPD Gel scoring pattern of different accessions of *P. pelagicus* using primer OPN-6.

Band size	Station I	Station II	Station III
250	1	1	1
300	1	1	1
400	1	1	1
500	1	0	0
750	1	1	1
850	1	0	1
950	1	0	1
1000	1	0	0
1250	1	1	1
1400	0	0	1
1500	1	0	0
1750	1	0	0
2000	0	0	0
3000	1	0	0
4000	1	0	0

Discussion:-

The development of molecular techniques has created new possibilities for the selection and genetic improvement of marine animals. The discovery of PCR had a major impact on the research of eukaryotic organisms and contributed to the development and application of various molecular markers [16]. The developments of DNA-based genetic markers have a revolutionary impact on animal genetics. DNA genetic markers have now been used for the molecular genetic characterization and genetic diversity studies in numerous species. Allozymes, mitochondrial DNA, RFLP, RAPD, AFLP, microsatellite, SNP, and EST markers are the popular genetic markers.

Genetic study of crustaceans is necessary to find out the best method of conservation and propagation of marine biodiversity. Genetic diversity refers to the variation at the level of heritable characters (polymorphism) and provides a mechanism for populations to adapt to their ever changing environment. The more variation, the higher the chance that at least some of the individuals will have an allelic variant that is suited for the new environment, and will produce offspring with the variant and will in turn reproduce and continue the population into subsequent generations. Genetic diversity comes in many forms. It can be measured in both individuals and populations. Genetic diversity can be haploid (DNA of the mitochondria), diploid, or even polyploid. Genetic traits can be based on the alleles at a single locus or many dozens of loci.

Two common terms of genetic diversity are allelic diversity and heterozygosity. Allelic diversity refers to the number of different alleles at any given locus in the population. Heterozygosity is the percentage of loci that are heterozygous in a population or individual [2]. A heterozygous locus is one in which the two alleles are different. When the alleles are the same, the locus is said to be homozygous. Both allelic diversity and heterozygosity are desirable. Allelic diversity is important for a population's long-term ability to adapt, while heterozygosity is important for more immediate individual health. When a new allele appears in a population, it has the potential to change the genetic make-up of successive generations. Harmful mutations will likely not persist because the affected individual will either not survive, or will have limited reproductive success. However, some mutations may be passed on to successive generations because an organism with that allele is better equipped to survive in its environment, that is, it has a selective advantage.

Genetic variation within population is caused by change of allele frequencies over time due to selection, random genetic drift and gene flow (immigration from or emigration to other population). Genetic variability is thought to be essential for the long-term persistence and adaptability of populations, and thus important in the management of captive and wild populations of endangered species. Reduction of genetic variability can reduce the ability of a species to cope with adverse environmental conditions, cause a reduced population density and, in some cases, lead to extinction of the species. When a population is greatly reduced in size, rare alleles in the population are lost if no individuals possessing these alleles survive [17]. The declining of rare alleles in the population causes overall declines of fitness in the population [18]. In the present study, population structure investigation of the species of crab *P. pelagicus* from three different geographical locations was carried out using RAPD PCR, a nuclear type II marker.

The blue swimmer crab is the most commercially important marine species in India and several countries in the world. This crab is extracted from the sea using traps and gill nets to meet the demand of the world market. *P. pelagicus* spread throughout the Indian and West Pacific oceans and it occupies sandy and sand-muddy in shallow waters between 10 and 50 m depth, including areas near reefs, mangroves, sea grass, and algal beds. The juvenile is commonly occurring in intertidal shallower areas [19]. In India, these crabs spread almost throughout the coastal island. Several key indicators show that *P. pelagicus* population is in crisis due to overexploitation.

In India, the population genetic structure of the blue swimmer crabs, *P. pelagicus* has not been well-studied unlike countries such as Australia and Thailand [20,21]. However, the increasing demands of *P. pelagicus*in the fisheries industry of India currently have led to a growing interest on the broodstock of this particular crab species. The knowledge of the genetic differentiation of *P. pelagicus* is no doubt useful for the effective management of this edible crab species with wide distribution range and long planktonic larval stages [21].

Arbitrary primers with higher GC:AT ratio have been proven to detect polymorphisms caused by point mutation [22]. The mutations will enable or disable the binding of a primer to the complementary sequence on DNA template. The bands are polymorphic if they are absent in some samples. This is an indication of the failure of primers to anneal in certain individuals due to difference in nucleotide sequences. In this study, polymorphisms have been detected

among individuals for both populations. This phenomenon was reported to be caused by the insertion or deletion between primary sites during amplification [23].

Genetic variability in RAPD analysis

A better understanding of population genetic structure is important to the effective fisheries management and conservation of genetic resources in exploited marine organism [24]. Stock enhancement and cultivation can reduce the probability. Admixed populations will undergo damaging genetic alteration in the event of a decrease in genetic variation, fitness, and effective population size, because maintaining the genetic diversity of admixed populations and their wild population components first requires managing both the genetic variability (e.g., numbers of alleles) and the genetic composition (frequencies of alleles) in the broodstocks and the broods [25]. According to [26], the major genetic risks of aquaculture include loss of genetic diversity within and among the populations and loss of fitness caused by the use of low genetic diversity and small numbers of brood stock. This situation can lead to inbreeding which in turn has implications for the quality of fry, a symmetrical survival of 'families' causing declines in stock quality The results of the present study will be helpful for improved management of crab stocks. Given the soaring value of the resource and decline of the stocks in several areas, all information that enhances management would be helpful. Prospective efforts to find genetic variation between various inshore and offshore crab populations should be attempted with high-resolution molecular markers.

Eventhough RAPD PCR is sensitive to numerous reaction factors, it is rather useful, when used with care, for a number of applications in marine animals. In the present study, the use of RAPD study to analyze the population structure of crabs along the Indian coast was established. Polymorphic fragments isolated through these studies could be used and probes be constructed which possibly will be helpful for single species detection. But, while interpreting the results of RAPD data, absence of amplicons / PCR bands should be cautiously interpreted. In this study, moderate levels of genetic variability were observed with crab species as revealed by nuclear RAPD markers.

In this study, RAPD profiles were generated from 3 geographically isolated crab (*P. pelagicus*) using four Operon primers. [27]recommended the use of six or seven primers to assess genetic diversity within and among populations of highly polymorphic species. Five primers were sufficient to reveal the stock structure in the crab *P. pelagicus*[28] from Thai waters. The primers used in this study were found to generate polymorphic pattern in the crab species. The percentage polymorphism (P) was almost similar in three populations. Of the four primers selected for study, three revealed clear polymorphisms except the primer OPA-19 which appeared invariant. RAPD analysis of 3 individuals of samples revealed different levels of genetic variability, also RAPD analysis of from three different geographical regions shows clear polymorphic patterns. The total number of polymorphic loci was 17 (65%). Genetic variation among populations did show evidence of divergence, especially with increased geographic distance. There was also evidence of adaptive phenotypic divergence among populations.

In this study, the RAPD phylogenetic tree shows that the populations were grouped into two definite clusters. It can be explained by their geographical separations of the Gulf of Mannar. Previous study by [29]summarized that the Atlantic horseshoe crabs also show clearly the population subdivision even in small geographic range, suggesting that there was deficiency of significant gene flow between populations studied by using mitochondrial cytochrome c oxidase I (COI) gene. The high degree of polymorphism and genetic distances on the population study showed that the genetic diversity of *P. pelagicus* life in Gulf of Mannar is quite high. The crab populations of Thoothukudi and Rameshwaram appear in one cluster, while the Kanyakumari populations formed the other cluster. Similar result was reported by [30] that genetic distance of *M. cephalus* from Gujarat, Maharashtra, Andhra Pradesh and Tamil Nadu in India varied from 0.3717 ± 0.1460 (Gujarat population) to 0.5316 ± 0.1720 (Maharashtra population). A dendrogram based on Nei's genetic distance also showed two clusters. The Maharashtra and Gujarat populations appear in one cluster, while the Tamil Nadu and Andhra Pradesh populations formed the other cluster. A high degree of polymorphism suggested a high degree of genetic variability between the samples [31]. According to [32] the presence or the absence of the polymorphism in PCR RAPD profile is either caused by nucleotide sequence divergence in primer sites or by insertions or deletions in the amplified segment of the template DNA.

Conclusion:-

The results of present study revealed that RAPD analysis from three different geographical regions showed clear polymorphic patterns. Thoothukudi and Rameshwaram populations appear in one cluster, while the Kanyakumari populations formed the other cluster indicating a genetic variability and diversity in samples collected from different places.

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