



Molecular Traceability of Spatial Genetic Diversity for Sustainability of Fish Genetic Resources: A Perspective for India

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Sustainability of aquaculture sector is considered pivotal for future nutritional security, to produce 40 million ton required from 2030 onwards. The management and improvement of fish genetic resources, holds the key to enhance production while overcoming the constraints of finite resources. In this context, it becomes imperative that the genetic variability in natural populations of aquaculture species is documented so as to plan its use in domestication, genetic improvement and for conservation of the natural genepool. A population of a species is composed of genetic stocks, which are locally evolving units and develop attributes of adaptive significance to the diversified local environments. Such intraspecific variability in the species can be a source of useful characters with potential application in domestication and genetic improvement of the species. The inadequate knowledge on genetic stocks of cultivable species of fishes is a major constraint in blue growth of aquaculture (CGRFA, 2016) and this is in contrast to the scenario in domesticated animals and plants, where breeds/ varieties etc are well documented. Therefore, bridging this knowledge disparity between fisheries and other agriculture sectors is necessary for implementing common guidelines on issues related to biodiversity, genetic erosion in farmed populations, IPR protection and technological advancements.

This presentation addresses a concept on the use of standardized molecular markers (Masih *et al.*, 2014) as traceability tools, to decipher the distribution and pattern of genetic variability. Such traceability tools can be applied to the wild and farmed populations, for varied objectives. The application of these tools to the wild population will discover the different sub-populations, needing strategies for *in situ* conservation, sourcing of the animals to implement improvement programmes. The known sub-populations can be evaluated, *on-farm*, for evaluation of production performance traits such as growth, reproduction, disease and any other adaptive trait

of economic significance. In a way, this could establish the knowledge of genetic stocks which exhibit variation in their phenotypes and production traits (as breeds in livestock animals).

In the aquatic species, phenotypic variation and production performance is not directly evident and may need indirect methods like scale reading for growth and image analysis for the phenotypes. However, results from such indirect analysis on the performance may be a comparative indicator of phenotypes but possibly not true reflection of their performance under controlled farmed conditions. Molecular markers could be useful to provide direct assessment of genetic divergence and the identified genetic stocks can be assessed for performance in culture. In a study carried under Outreach project on Fish Genetic Stocks, Nuclear (Microsatellite DNA) and partial mitochondrial genes (Cytochrome b and ATPase 6/8) were analyzed for wild populations of Indian carps and catfish and indicated the existence of genetic divergence in wild populations. An example of spatial distribution of genetic variability in wild population of two species, *Labeo rohita* and *Clarias magur* with contrasting biology is presented here. *Labeo rohita*, a carp, is native of rivers in Indo-gangetic region and found and dispersed in fast flowing rivers. *L. rohita* is the icon of Indian polyculture. In the contrast, catfish *C. magur* lives in stagnant puddles and may be dispersed during floods. The *Labeo rohita* (N=925, 19 localities) samples were collected from four different river basins, include Indus, Ganges, Brahmaputra of Indo-gangetic region and Mahanadi river system flowing through peninsular India. For *C. magur* (N=792, 16 localities) were collected from northern, western and peninsular India. In the two respective species, all the three class of markers used, provided similar results. *Labeo rohita* population had low genetic differentiation (>8%) while high genetic differentiation (26%) is seen in the *C. magur* population. The Bayesian analysis on spatial scale did

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not confirm existence of river wise genetic stocks in *L. rohita*, rather admixture of divergent clusters in different rivers was evident. This admixture may be the result of ancestral genotype or gene flow happening in continuous river systems. In *C. magur*, high genetic differentiation was evident and spatially differentiated clusters were existing.

The identified genetic clusters based on standardized molecular markers become appropriate candidates for aquaculture improvement programmes.

On the other hand, the domesticated populations in aquaculture (Indian major carps and shrimp in India), possibly held with small-scale farmers or hatcheries, which could possibly have differentiated genetically from their wild ancestors, need to be identified on spatial scale. Such farmed populations after several generations of domestication might have lost some alleles or acquired new useful alleles. The ignorant management practices or use of unauthenticated broodstock (as considered in shrimp) might cause genetic erosion and high propensity of disease risks. The traceability will help to develop framework of policies and guidelines, with an objective to preserve genetic diversity than eroding it. This will open up new avenues for the preservers of diversity, to trade the genetic products (such as improved seed, broodstock) at national, regional and international level. Trading of genetic products is happening in livestock but not so prevalent in aquaculture yet. However, the spread of *Litopenaeus vannamei* (white leg shrimp), showed that this is possible as broodstock worth 50 million USD was traded from Hawaii, during 2015. This presentation proposes a concept, "The GRA (Genetically Responsible Aquaculture) plan": in this context, with an aim to policy change for preserving aquaculture genetic diversity.

'The GRA plan' is novel and yet not tested, nevertheless, system changes envisaged here, is a necessity for sustainable intensification of aquaculture. The scenario emerging from the Asian shrimp culture in the last two decades, also indicates this lesson. The authors were encouraged to frame this plan with the letters from two shrimp farmer associations in India to NACA (Network of Aquaculture Centers in Asia-Pacific), recognizing that the shrimp post-larvae needs innate fitness, often eroded, when unknown pathogens co-exist in pond culture post SPF (Specific Pathogen Free) stage. The GRA plan will enable formation of farm genetic-exchange networks (Doyle *et al.*, 2015),

novel for aquaculture, but resembling farm associations for terrestrial crops and livestock sectors, that conserve genetic diversity, indefinitely within regional "meta-populations". Misrepresented, diluted and inbred seed is threatening the livelihoods of small-hold aquaculture in Asia-Pacific. Protocols using novel, genetic-marker-based "certificates of authenticity" will be developed to prevent misrepresentation, while opening new global opportunities for small enterprises to use genetic diversity for trading new genetic products and commodities (e.g. certifiably "fair trade").

In this endeavour, three aspects critical for implementation of the plan are:

1. *Traceability Protocols and Genetic Diversity Indices:* Demonstrating that the traceability tools and genetic diversity indices assays, are available, performed easily in routine laboratories and low in expenses, the farming community is likely to accept. Development of tools is critical and can be overcome, though initial molecular research could be intensive. It needs to establish standardized species-specific marker set with the reference levels of genetic standards. This standard marker set protocol can be improvised to accomplish in the existing diagnostic facilities used in shrimp aquaculture. This will enable the wide use of genetic standards assessment tools in seed and broodstock, verifying origin, strategizing breeding plans and securing the fair trade. This can provide rapid appraisal of farm-level inbreeding and regional genetic diversity and help in avoiding using broodstock and stocking seed from inbred sources. This can contribute to a good disease mitigation with genetic fitness and support the existing pathogen control mechanisms like SPF protocols.
2. *Empirical Data on Magnitude of Genetic Erosion, Consequent Production Performance Losses and Linkage with Socio-economic Impact on Small-scale Farm-holds:* This critical information is vital to press for a systematic improvement in aquaculture broodstock and seed management system in developing countries. This will facilitate taking steps for implementing the new procedures as part of GAP standards. The benchmark survey and analysis in this start-up proposal (in selected regions) will give an insight on the genetic erosion and resulting income losses due to underperformance of seed, disease susceptibility and unfair trade practices due to misrepresented seed and broodstock. This will be

linked to socio-economics, impact on woman and children, in farming house-holds.

3. *Information Management for National/Regional Broodstock Improvement Banks or Network*: National Broodstock Bank or Network (NBIN) is a knowledge driven strategy for long-term preservation of on-farm genetic diversity, maintain genetic gains, and establish informed breeding plans and exchange of genetic material. Information management will be a crucial element in developing and sustaining such operational networks. This could be spatially distant farms linked through establishing IT based database, accessible through single nodal point maintained regionally or nationally and provide genetic information of the broodstock available.

Conclusion

In view of the above, standardized molecular markers for cultivable species are important and need to be established with the genetic indices. This can be used for varied objectives of knowledge development for use

in genetic management of wild relatives and genetic fitness of farmed populations.

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