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Genetic tools and techniques for fish improvement

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Abstract

The Indian continent is a rich source of marine, freshwater, brackishwater and cold water species of fishes of which more than 2900 species have been documented so far. India is blessed with a long coastline of 8129 kms, inland water bodies like rivers and reservoirs, lakes etc. Fisheries and aquaculture in our country contribute about 1.5 % to the GDP. In addition to providing food and nutrition security to millions of people, about 14 million people are directly or indirectly employed in this vibrant sector. Response to selection for body weight in first is higher because of existence of high genetic variability. The fecundity is also very high in fish/shellfish thereby allowing for a higher selection intensity.External fertilization offers a great opportunity for production of family as per choice. Fisheries research especially related to genetics had been initiated by the ICAR in a very systematic manner. Genetic characterization of candidate species has also been undertaken in a major way since 2008. Microsatellites and mitochondrial genes ATPase 6/8, Cytochrome oxidase I, 12S rRNA and 16S rRNA are also being used for genetic characterization. Individual selection is still considered the best choice although family selection could also play a vital role in retaining genetic variability. ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar along with the Norwegian Agency for Development Cooperation (NORAD) initiated the selective breeding programme on the carp-*Labeo rohita* in 1992. This is the first genetic improvement programme on fishes in our country and till date is in vogue with substantial genetic gains in growth. In 2007, at the same institute, genetic improvement programmes in the giant freshwater prawn *Macrobrachium rosenbergii* and recently on another carp *Catla* was initiated. Fish genetics study at ICAR-Central Institute of Brackishwater Aquaculture, Chennai and ICAR-Central Institute of Fisheries Education, Mumbai in collaboration with the NORAD on the tiger shrimp *Penaeus monodon* revealed that growth has moderate to high heritability and individual selection is the preferred choice for eliciting maximum response. Additive genetic variances have been exploited to control some diseases, whereas in White Spot Syndrome Virus disease in shrimp, the additive genetic variance is negligible, thereby making selection ineffective. Presently, a genetic improvement programme on the fgreshwater catfish *Clarius magur* has been initiated at ICAR-CIFE at Balabhadrapuram near Kakinada

Key words: Brackishwater aquaculture, selective breeding, fish species, shrimp

Introduction

Fish is a very important source of nutritious protein for human beings. Fishing has been an activity ever since mankind started hunting in the olden days. Fish can be caught from the sea (marine), lakes, rivers and reservoirs (fresh water) and from brackishwater (salinity less than that of seawater : < 35 ppt). Brackish water is where the rivers meet the sea (like in an estuary) and there is a dilution of salinity. The aquatic animals found in brackish water are continuously in a state of stress because of the fluctuation of salinity. Aquaculture production is very much different from either the plant, crop and animal production systems. Aquatic animals live in water and all physiological processes occur under water. This makes the process of growth, reproduction and disease tolerance in water a very complex affair. Compared to terrestrial farm animals, aquatic species are very difficult to work on and their requirements are also very specific. The requirements among freshwater species, brackish water species and marine species are again different, which makes the proposition of aquaculture production management very complicated.

Status of global and Indian fish production

The global fish production has been increasing over the last decade and in 2016, the fish production was

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171 million metric tonnes which includes finfishes, crustaceans and molluscs. The capture fisheries was 90 million MT and that from aquaculture was 80 million MT. Out of this 171 million MT, 150 million MT goes for human consumption. (FAO 2018). Globally, in 2016, 40.3 million people and 19.3 million people were engaged in capture fisheries and aquaculture respectively thereby contributing substantially to generation of employment. In the same year, 85 percent of the global population engaged in the fisheries and aquaculture sectors was in Asia, followed by Africa (10 %) and Latin America and the Caribbean (4 %)[FAO,2018]. By going through these statistics, one can very well comprehend the importance of fisheries and aquaculture in employment generation. Fish production in India hovers around 10.7 lakh tonnes of which 47% is from capture fisheries and the remaining 53% from aquaculture (www.nfdb.gov.in). India is at the fourth slot in fisheries production after China, Indonesia and USA whereas in aquaculture our country ranks third, after China and Indonesia (FAO, 2017). India in 2017-18 exported 13.7 lakh tonnes of fish/shellfish valued at Rs.45,106 crores (www.mpeda.gov.in). This accounts for around 10% of the total exports of the country and nearly 20% of the agricultural exports. More than 50 different types of fish and shellfish products are exported to 75 countries around the world. Shrimp contribute about 41% by volume and 69% by value through exports. (www.mpeda.gov.in). India is bestowed with a variety of more than 2300 fish species. In freshwater aquaculture, carps contribute substantially. Aquaculture and fisheries are a vibrant sector in our country. Fisheries and aquaculture contributes 1.5% to the GDP and 5.1 % to the agricultural GDP. In addition, it provides food and nutritional security to millions of people and generates direct/indirect employment to 14 million people. In India, we have eight research institutes under the Indian Council of Agricultural Research which are mandated to carry out research in different species of fish, be it freshwater, brackish water, coldwater and marine.

Aquaculture species vs farm animals

There are some aspects in aquatic species which are very much advantageous for selective breeding. The coefficient of variation for growth in terrestrial animals range from 7 to 10% whereas it ranges from 20-35 % in aquatic animals indicative of more variability**.** It also indicates that selection will be very effective in improving characters. Also, the fecundity in aquatic animals is very high. For example, in the crustacean *Penaeus monodon* (tiger shrimp) a female shrimp would produce about 2 to 4.5 lakhs eggs per spawn. Tilapia a finfish, yields 300 to 1500 eggs per spawn. In fish, a high selection intensity could be practiced, as there is a large number of young ones from which we could select individuals for breeding. The fertilization is external which makes it greatly flexible to produce half-sibs and full-sibs. The production of breeding animals in fishes is cheaper compared to terrestrial farm animals. However, there are also some disadvantages. At hatching, the individuals are very tiny and are impossible to tag. For example, in shrimp, the juveniles can be tagged only when they reach a size of about 3-4 g. Consequent to this, each family has to be reared separately till tagging and this becomes very expensive. However, we can also use microsatellites (genetic tagging) but the procedure is quite expensive. In closed populations, there would be a rapid buildup of inbreeding. The traits that are amenable for selection in aquatic species are growth rate, disease resistance, feed conversion efficiency, meat quality traits, age at sexual maturity etc. Aquaculture production can be increased by domestication and development of genetically improved stocks. Breeding programmes need a lot of investment both in terms of infrastructure and labour. In addition, there should be a cohesive group of scientific and technical personnel who are trained in quantitative genetics, aquatic animal health and fish nutrition. In addition, only a consortium approach like a public private partnership would yield the desired results. The output would be such that it benefits the farmers, the industry and finally the nation as a whole.

Selective breeding programmes in aquatic species

Globally there are many selective breeding programmes in Atlantic Salmon, Rainbow trout, Nile Tilapia, Rohu, Asian seabass and the Pacific white shrimp. In India, genetic improvement programmes are in place for carps like rohu and catla and giant freshwater prawn or scampi. The genetic improvement programme on rohu was initiated at the ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar in 1992. This was a collaborative project with the AKVAFORSK, Norway. Growth is the trait selected for and resistance to aeromoniasis was added later as an additional trait. There has been a genetic gain of about 18% per generation after 9 generations of selection and the selected strain is named as Jayanti Rohu (Mahapatra et al. 2016). Similarly a programme has been initiated recently in Catla. In crustaceans, the giant freshwater prawn *Macrobrachium rosenbergii* has been subjected to selective breeding at ICAR-CIFA (Pillai et al. 2017) under a World Fish Center assisted collaborative research project. After selection, the response till generation 9 was 7% per generation. A genetic improvement programme on the fgreshwater catfish *Clarius magur* has been initiated at ICAR-CIFE at Balabhadrapuram near Kakinada. The response to selection in growth has been carried out for one generation and the results are encouraging. In shrimps, selectively bred and specific pathogen free (SPF) Pacific White shrimp *Penaeus vannamei* is available in USA as well as in Latin America. Hatcheries in India are legally importing the SPF *P. vannamei* from abroad, following the necessary guidelines of the Coastal Aquaculture Authority (a regulatory agency tasked with the responsibility of overseeing the shrimp culture of our country), producing seeds and selling them to the farmers who culture them. The farmers culture the shrimp and most of the shrimp produced is exported. The MPEDA under the Ministry of Commerce & Industry of the Government of India is the agency entrusted with the export of capture fisheries and produce from aquaculture especially shrimps.

Genetic studies on tiger shrimp

From 2004 to 2009, the ICAR-Central Institute of Brackish water Aquaculture, Chennai along with ICAR-Central Institute of Fisheries Education, Mumbai and AKVAFORSK, Norway had carried out a collaborative research project on the estimation of genetic parameters in the tiger shrimp. This study reported genetic parameters in tiger shrimp for different traits for the first time in our country. The results revealed that growth and pond survival could be improved by selection (Krishna et al. 2011) whereas there was limited genetic variation for resistance to White Spot Syndrome Virus (WSSV) (Hayes et al. 2010). It also revealed that genotype-environment interaction for growth was not important for tiger shrimp. It was also observed that there was a reproductive bottleneck in tiger shrimp as the breeding efficiency was very low. Later from 2009 to 2013, there was a concerted attempt through a collaborative research project between ICAR-CIBA and Norwegian Institute of Food, Fisheries and Aquaculture (NOFIMA) Norway, wherein the transcriptome of tiger shrimp was brought out. This was followed by a challenge test for unravelling QTLs for resistance to WSSV. The study revealed 9 QTLs for resistance to WSSV and also a linkage map using SNPs was constructed for the first time globally. (Robinson et al. 2014; Baranski et al. 2014). All the nine QTLs were observed in genes that were connected to immunity. In addition, for the first time globally, a chip containing 6000 SNPs was prepared using Illumina platform which was used to genotype the shrimp for unravelling the QTLs for resistance to WSSV.

Genetic tools used for fish improvement

The ICAR in 2008 initiated an Outreach Activity project on genetic characterization of fish genetic stocks where all the important candidate species of fish were identified for different institutes. The results have been documented. This study is a prelude to any genetic improvement programme. The analyses of mitochondrial genes was used for this purpose. For finfishes, ATPase6/8 was utilized whereas in shrimps, Cytochrome b, 16S rRNA and control region were used. For mud crabs, 12S rRNA and 16S rRNA were used. In addition, truss morphometry was also used to discern shape differences.

Growth in aquatic species is a trait exhibiting a heritability of more than 0.2, hence individual selection is utilized for eliciting response. In fishes, combined selection is also carried out. The individuals to be used as parents for the next generation are selected based on their breeding values.

Tagging is a process wherein individuals are identified. In fishes, we use the Passive Implant Transponder tags (PIT tags) which are injected into the muscle of the fish and can then be read using a reader. In crustaceans, tagging is a lot more complex as they shed their exoskeleton quite frequently. Hence in shrimp we use the Visible Implant Elastomer (VIE) tags, in the giant freshwater prawn we use the visible implant alphanumeric (VIA) tags and in crabs we use coded wire tags. These tags are inserted into the muscle and can be seen/identified from outside. Even though the crustaceans molt, the tags remain intact. In shrimps, VIE tags are used for family identification whereas eye-ring tags are used for individual identification.

Pre-requisites for a breeding programme in aquatic species

The first aspect is the standardization of maintenance of brood stock in captivity, followed by reproduction. It is extremely difficult to domesticate an aquatic species as it takes years to do so. The reason is that the environment in captivity is totally different from that in the sea. Closing of life cycle is the foremost condition for starting any genetics experiment. Breeding efficiency needs to be around 80-90 %. The sexes should be discernible at early stages. Tagging needs to be done at an early age. It has also been observed that fish require a natural ecosystem i.e. ponds for the proper expression of a trait especially during grow-out culture.

Conclusion

Genetic improvement studies in aquatic species are very few in number in our country. As on date, there are only three such programmes in place at the ICAR-CIFA, one in the giant freshwater prawn and two in carps. Compared to terrestrial farm animals, selective breeding in aquatic species is very difficult. The coefficient of variation observed in growth in aquatic animals is comparatively high indicating that this trait would be amenable to selection. Genetic characterization of important candidate species has been documented. Genomic studies on certain selected species like Indian white shrimp is in progress at ICAR-CIBA, Chennai. The information generated from genetic characterization as well as from genome sequencing would ultimately be utilized for genetic improvement programmes. Once their life cycles are closed, important candidate aquaculture species need to be subjected to selective breeding programmes so that genetic gains can accrue and the selected strains can be available for farmers to carry out the culture. A lot of deliberations have to take place between the research institutions, fish farmers and industry personnel for the choice of candidate species. Culture of selected strains of fish/shellfish can result in enhanced production which in turn would increase the profits for the fish farmer.

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