Fish Technology

Genetic Improvements in Aquaculture industry:

In the transition from wild harvest of a product to agricultural production, genetic improvement is always an important developmental step. The first stage is usually the process of domestication. Further genetic improvement in agriculture or aquaculture typically has a human focus on enhanced production characteristics.

Just as in terrestrial production scenarios, aquaculturists value faster growth, improved efficiency, less loss to disease, and high quality. Several tools are available to accomplish such genetic improvements.

Domestication

Most stocks of the aquatic organisms most widely grown in captivity are domesticated to some degree, and a few have undergone further genetic improvement. Just as terrestrial livestock and crops are bred to be not only productive, but productive in convenient proximity to humans, aquatic plants and animals are now undergoing these same pressures.

Although the domestication process is arguably at a much earlier stage for aquatic organisms than their terrestrial counterparts, aquaculture is undergoing the process at a time when the technologies and tools available could remarkably speed up domestication and genetic improvement.

Selective breeding

The basic tool available for genetic improvement, selective breeding, entails choosing the animals with the highest genetic value as breeders for the next generation. To determine this genetic value, populations are raised in a standardized environment so identified differences will be due to differences in genes, not the environment.

This sort of testing is expensive in terms of the facilities required to standardize the environment, maintain consistent rearing, and measure hundreds to thousands of groups. The elite performers are selected for breeding to produce subsequent generations.

This “classical quantitative genetics” approach has been around since the early 20th century, and most of the genetic gains realized through today can be attributed to traditional selective-breeding methods. Yet to think this branch of science has remained static would miss the mark greatly. Improvements in experimental designs, tagging technologies to identify individuals and groups, and computational tools have revolutionized the accuracy and power of these classical techniques.

Genetic markers

Genetic markers include several classes. Amplification fragment length polymorphisms and microsatellite markers are two of the most frequently used genetic markers in aquaculture. They reflect regions of DNA that are used to keep track of specific locations within the genome. The markers are distributed throughout the DNA of an organism and transmitted from parent to offspring.

Genetic markers are useful for determining parentage because offspring share markers with their parents. They are also expected to be useful for identifying regions of DNA that confer performance advantages.

If a particular marker is always found associated with resistance to a coldwater bacterial disease, for example, selection for breeding could be based on the presence of this marker without doing a challenge trial under tightly controlled environmental conditions. This application of selective breeding is referred to as marker-assisted selection.

An important consideration for marker-assisted selection is that traditional approaches relying on direct trait measurements in organisms are expensive. Furthermore, elite performers in disease challenges are usually not used for breeding for fear of transmitting disease, but their close relatives could be used.

A similar situation occurs if traits of interest are measured after test animals are slaughtered. Individuals with high meat quality or fillet yield may not be available for breeding after the traits are measured.

Genetic engineering

Genetic engineering is a broad heading that includes chromosome set manipulations and transgenesis. Chromosome set manipulation is a group of techniques in which sets of chromosomes in organisms are modified.

Whereas normal organisms have two sets of chromosomes – one from each parent – one type of chromosome set manipulation can result in triploids, organisms with three sets of chromosomes. These animals are generally sterile, which can be advantageous under conditions where reproduction is undesirable.

This technology has been applied in the United States to the nonindigenous weed-controlling grass carp to prevent unwanted reproduction. Chromosome manipulation has also been used in oysters, which typically undergo a loss of quality during the spawning season.

Transgenesis

Transgenesis refers to the technique of incorporating a gene or genes through biotechnological methods, not breeding. Soybean plants that have been made resistant to the effects of herbicides are an example of transgenic crops. With a gene from bacteria transferred into and expressed in

the plants, their use reduces labor costs for cultivating and weeding. Golden rice, a transgenic rice plant, has three foreign genes inserted from bacteria and daffodils to allow the production of vitamin A in the rice.

The Glofish, a fluorescent zebra-fish which came on the U.S. market in January 2004, is the first transgenic aquatic organism to be marketed. The proteins responsible for making the zebrafish fluoresce are transgenes derived from jellyfish and coral. These fish are not intended for food.

Atlantic salmon with a growth hormone gene from Chinook salmon and a promoter, or switch, from the ocean pout are currently being reviewed by the United States Food and Drug Administration for approval as food fish. Research on other aquatic transgenics with the potential for greater disease resistance, enhanced nutritive value, and other characteristics is ongoing around the world.

Induced Breeding in fish:

Major carps are most important species from the point of view of their high food and nutritive values. Hence they have kept attention of scientists and aqua farmers. They have peculiar habit of breeding in running waters of rivers and streams where they have large space for movement.

During breeding season, water is sufficiently provided with minerals, O_2 and food contents. This friendly aquatic environment provides stimulus for spawning. Carps do not breed in confined water of captivity even if their gonads are matured and ovulation might have taken place in natural environment. For the increasing production of carps it is necessary that they should be made to breed in confined water so that increasing demand of good quality fish and their seed could be available. This can be done by adopting induced breeding technique by which ripe or mature fishes breed in confined water when stimulated by injection of pituitary hormone. The pituitary hormone is an important gonadotropin, which is extracted from the hypophysis of a mammal or a mature fish.

Induced breeding of carps in captivity by the use of pituitary hormone injection has been successfully done. Many scientists are involved in doing research to improve induced breeding technique. Several attempts are being made to establish pituitary banks to meet out the need of pituitary hormones throughout the year.

Steps of Induced Breeding Technique:

Induced breeding technique has following steps:

1. Collection of Pituitary Extract:

Pituitary gland is collected from a mature fish, which is called as a donor fish. Most widely used donor fish is the common carp, Cyprinus carpio. The best time for preparation of pituitary extract is May and June. The Indian major carps like Catla catla , Labeo rohita, Labeo kalbasu, Labeo gonius and Cirrhinus mrigala do not breed in confined water and have need to be subjected to induced breeding.

To remove pituitary gland, the head of the fish is dissected and brain is exposed. The gland is immediately removed from the brain and stored in a refrigerator. It may be preserved in absolute alcohol at room temperature.

The gland then is homogenized in distilled water. The homogenate is centrifuged and clear supernatant is used as source of hormone to which 0.3% sodium chloride solution is added to it. This extract is ready for the immediate use. If pituitary extract is to be stored for a longer period the glycerin or trichloroacetate acid may be used instead of sodium chloride.

2. Selection of Breeders:

Medium sized fully ripe and healthy fish of around 2 to 4 years of age is preferred for induced breeding. The weight should be 1 to 5 kg. Healthy male and female breeders should be identified and netted out before the breeding season and should be kept in spawning pools. They should be provided with supplementary food.

3. Injection of Pituitary Extract:

To ensure higher success rate of fertilization it is important to coincide time of ovulation with the release of milt of male fish. For this purpose usually ratio of female and male 2:1 is maintained

in every set. Dose of pituitary extract to be given is decided according to age, sex, weight size and state of maturity of both donor and recipient. A dose 2 to 3 mg of gland per kg body weight is given to female breeder.

There is no need of injecting dose to the male breeder if it in a state of milt oozing. After 6 hours of the first dose of injection another dose of 5-8 mg of gland per kg of body weight may be given to female if needed. However, a dose of 2-3 mg per kg body weight is recommended for the male breeder. More than 2 injections should not be given.

The injection given may be intra-macular at caudal peduncle or shoulder or intra-peritoneal at the bases of paired fins. The first injection should be given at the early hours of the day, while the second one in the evening. Weather should be rainy or cloudy for easy and early spawning. The fishes should be transferred to the breeding hapa after injecting the pituitary hormone.

4. Spawning in Breeding Hapa:

A pair of breeder is released into the breeding hapa for spawning after injection of pituitary extract. The breeding hapa is a rectangular case of fine netting. For larger fishes its size is 8′ x 3′ x 3′, but for the smaller fishes it is 5’x 3’x 3′. It is held on four bamboo poles, one at each corner of the rectangular case. The roof of the hapa may be open or closed.

The hapa is made of mosquito net cloth through which laid eggs and milt cannot escape out. Three-fourth part of hapa is summarized in water whereas upper one-fourth part remains in air. After 3 to 6 hours of injection of pituitary extract spawning takes place.

The fertilized eggs are white and opaque whereas unfertilized eggs are transparent and bead-like. A hatching hapa is also rectangular and made of muslin or malmal cloth and is open from above. The mosquito net hapa is present inside the hatching hapa.

5. Precautions for Induce Breeding:

(1) To avoid diseases and parasitic infections, breeders should be properly washed with $KMnO_4$ solution (0.5 g in 100 litres of water) for a few minutes. After this they should be kept in formalin (200 mg/ It of water) for one hour.

(2) Breeder should be protected from mechanical injuries during handling.

(3) Water condition should be favourable having temperature about 24 to 31°C and turbidity about 100 to 1000 ppm. Flowing water with higher O_2 content is of great use. The intensity and duration of light also affect the induced breeding and spawning. Pituitary glands taken from the same or related species as the recipient species are said to be more effective.

Advantages of Induced Breeding:

1. The seed spawn is timely available, its availability from natural sources is quite uncertain.

2. A pure spawn of a desired species is made available. The spawn obtained from the rivers are not pure. They are mixed with the spawns of other species and sorting of pure seed from the mixed spawn is not possible.

3. Any quantity of pure spawn can be made available.

4. Several carps attain sexual maturity in ponds but they do not breed in confined water. Such fish can be subjected to induced breeding and spawn can be collected.

5. It is economical to obtain a spawn from induced breeding experiments in comparison to its collection from the riverine sources.

6. The induced breeding technique is very simple and can be learnt even by a layman.

Transportation of fish seed:

Transport of seed from natural environment or hatcheries is considered to be a crucial step in aquaculture. Traditional methods of carrying the seeds in earthern pots called ‘’Hundies’’ but this result in high mortality. υ Seeds being active, this results in exhaustion of available O2 of media at a shorter time. They need to be anaesthetized to reduce the activity to transport them in good condition.

Important parameters to be considered before transportation –

1. Oxygen requirements.

* The oxygen required by spawn in mg/gm body weight is 10 times greater than fry and fingerlings.

* The tolerance ratio is calculated by increasing the oxygen level and comparing it with the CO2 level.

2. Oxygen consumption rate.

* The rate is proportionate to its size or body weight.

* ie, if the length is different and weight is same the O2 consumption will also be the same.

3. Ammonia and rate of O2 consumption.

* Spawn can tolerate 2.5ppm dissolved free ammonia and 15ppm of dissolved ammonia as inorganic salts.

* Increase in ammonia will results in decrease of O2 and increase of CO2 in blood.

4. Temperature and O2 utilization.

*Metabolic activity is directly proportional with temperature.

Reasons of mortality

- Dissolve oxygen level in transporting water reduces and Carbon dioxide level increases.
- Due to metabolic activity of fishes the concentration of ammonia, urea and uric acid etc increase in the water, hence fish gets stress.
- If transportation is done in high density it may lead to mortality of fish due stress of oxygen.
- If transportation is done in improper vessels, physical damage of fishes may occur.
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Methods of transporatation

Mainly two methods:

1. *Open method*

   a. earthern pots.

   b. aluminium pots protected externally by coir mesh.

2. *Closed method*

   Plastic bags, buckets, collapsible plastic pools and fibre glass tanks.

Open system -

- The containers are carried on sling along small roads and paths to deliver.
- In open method water is continuously splashed or agitated for aeration. This makes the seeds more stressed and leads to mortality.
- Stressed seeds become more inactive and are subjected for predation or injuries.

Closed method

- In closed system fiber glass containers or plastic bags are commonly used.
- The seeds are conditioned before collecting and are oxygenated using cylinders.
- Containers are filled 2/3 oxygen and 1/3 water .
- Fishes are first conditioned by starving them and keeping them in a crowded condition.
- The plastic bags are kept in light tin containers or cardboard cartons and transported long distances by road, rail or air.

Seed collection and conditioning

- Conditioning actually means preparing the fish seeds to remain in a hardy condition.
- First, the seeds are collected by nylon / cotton cloth and dragged through water to remain in a smaller area to create a crowded condition.
- The fishes remains in this condition for a few hours to void their gut content.
- As size of fish increases the time of conditioning also increases.
- While keeping them in this, water is splashed into this small area to fasten the gut content elimination process.

  * The fry - minimum of 3 hours.
  * Early fingerlings (35-50 mm) for 6 hours,
  * Advanced fingerlings (80-100 mm) for 9 hours
  * Juveniles (150 mm) for 12 hours.

Preparation of packing the seeds

First the seeds are bathed either in potassium permanganate (2-3ppm) or common salt (0.3%) for few hours.
Process:
* Check the plastic bags for any leakage.
* keep them in clean tins provided with a lid to close it.
* Put pieces of used newspaper between the bags and the wall and the bottom.
* fill the bags with water taken from where the seed is taken.
* The seed are packed in plastic bag 1/3 full of water and 2/3 full oxygen tied with string and keep securely in tins.
* the tins should be transported in shades.
* transportation should be in morning or evening.

Anaesthetic

- In aquaculture, anaesthetics are used during transportation to prevent physical injury and reduce metabolism (DO consumption and excretion).
- An ideal anaesthetic should induce anaesthesia rapidly with minimum hyperactivity or stress.
- It should be easy to administer and recovery should be rapid.
- The anaesthetic should be effective at low doses and the toxic dose should greatly exceed the effective dose so that there is a wide margin of safety.

Characteristics needed for anesthetics

- Must be water soluble.
- Dosage required should be low.
- Time of induction and recovery should be short.
- Fish will tolerate well for several hours at low concentration.
- Should not have any side effects in the fish.
- Lethal concentration should be high, so that fish do not die accidently.

Anesthetizing and Transportation

- After conditioning fish seeds to be anesthetized to reduce the activity thereby reducing mortality. Anesthetics are chemicals used to reduce the metabolic activity of fish seeds by depressing the activity of brain.
- This will leads to reduce the O2 consumption.
- Concentration of usage depends on the size, shape and age of fishes.

Determination of quantity of fish

- Quantity of fish seeds for transportation depends on their size, mode duration of transportation, salinity of the medium and the ambient temperature.
- Formula to find the number of fish seeds:

$$N = (DO-2) X V C X h$$

Where.

DO= dissolved oxygen in ambient water in mg/l

V= volume of water in litres.

C= rate of oxygen consumption by the individual fish (mg/hr).

h= duration of transportation (hr)