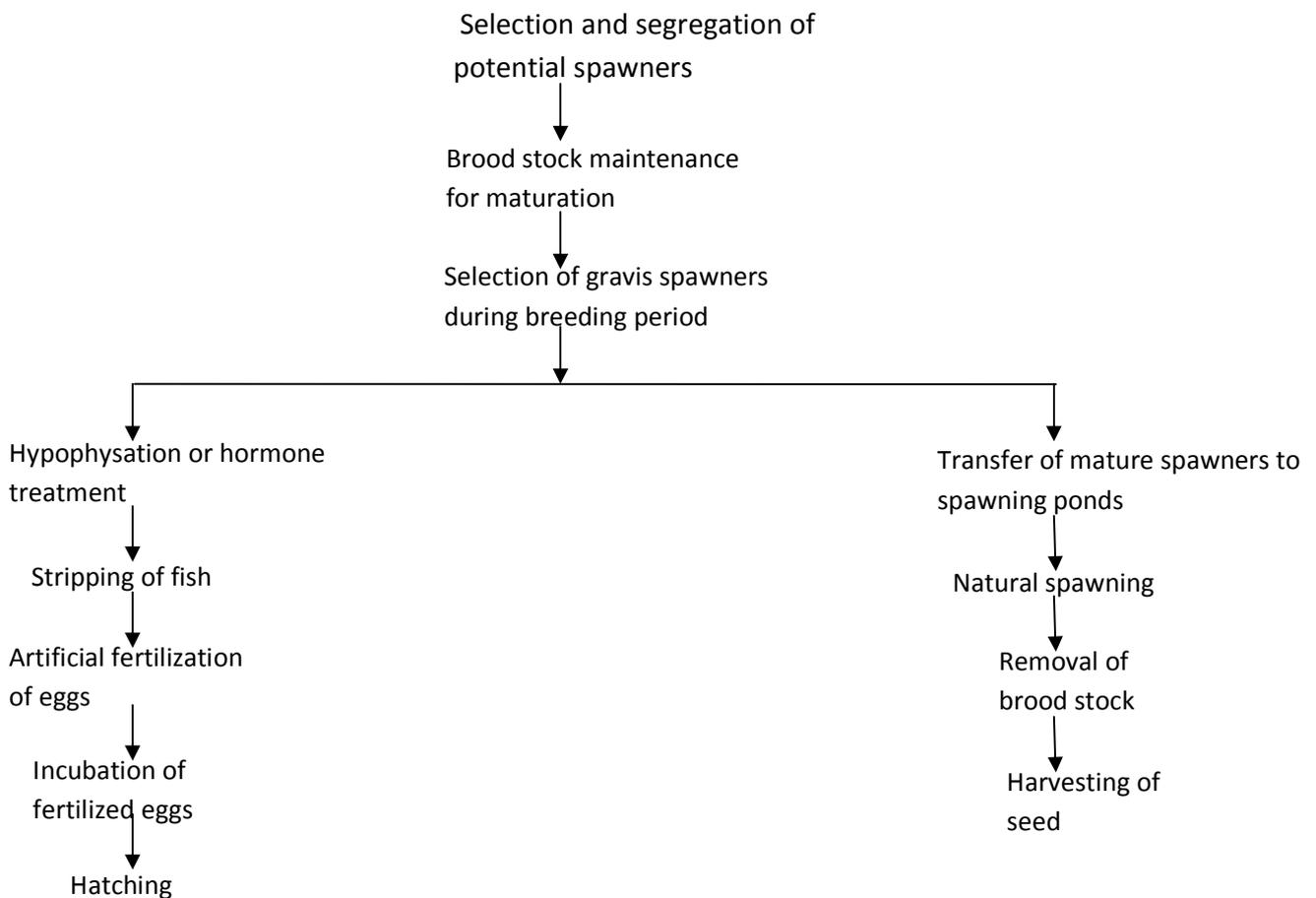


## FIS313

### ARTIFICIAL FERTILIZATION

Artificial production of fish seed involves human intervention in the natural propagation processes. This may be achieved by creating appropriate environmental conditions which are conducive to the spawning of the species. More commonly, the fish may be artificially induced to ovulate and spawn through injection of natural and synthetic reproductive hormones. Artificial production of fish seed is beneficial to the fish farmers in that the juveniles of the required species can be produced as when required, irrespective of the quantity and the time of the year. It also makes possible the production of seed from good quantity stock and hybrids of desired species. The processes of artificial production of fish seed are carried out in enclosures known as hatcheries which may be an indoor or outdoor facility and require various inputs like brood stock adequate water supply and suitable feed. The sequence of steps involved in artificial production of seed is as follows:



**Sequence of steps in the artificial rearing of fish seed**

## INDUCED OVULATION AND SPAWNING

In culture conditions, most fishes need to be induced before yielding their gametes during sexual reproduction. Two techniques can be used:

### (1) Induced Spawning by Stimulation of Appropriate Environmental Conditions

Provision of natural nesting materials such as grasses in the littorals and slowly rising water level will stimulate nest building and subsequent spawning in *Heterotis* and *Gymnarchus*. The most frequently used artificial spawning substrate used for common carp, *Cyprinus carpio* and the mudfish, clarias species, are known as “kakabans”. These are mat-like structures made of polythene fibers, pine tree branches or similar, materials each measuring up to several square meters in area. They are spread on the pond floors and anchored with sticks or suspended about 20 – 30cm below water surface. Small drums, cut bamboo stems and PVC pipes cut in one meter length and sealed at one end are required to act as spawning receptacles for brooders such as chryichthys species in ponds.

### (2) Inducing Ovulation and for Spawning by Hypophysation

In meter, ovulation in a fish is regulated and brought about by the gonadotrophic hormone produced and stored by the fish’s pituitary gland. The stored hormone is released into the blood when all requisite external and internal conditions become favourable. In hypophysation technique, gonadotrophic hormones extracted from the pituitary gland of another fish called the donor, is administered to the brooder. At appropriate dosage, this may bring about the ripening of the fish gonads and trigger spawning activities. Hypophysation can also be achieved by using artificial preparations containing human chorionic gonadotopin (HCG) or synthetic cortico-steroids e.g. decontrasterone acetate (DOCA) dissolved in glycerine.

One to two milliliters of the extract is carefully injected into the dorsal muscle or the abdominal cavity of the fish. Extracts from the pituitary glands or ripe fish of the same species with matching weight are known to be most effective, though carp pituitary extracts are effective on several other especially salmonids and catfish.

## CARE OF BROOD STOCK

Sexually ripe and healthy fish are the pre-requisite for any kind of artificial or semi-artificial propagation. They can be obtained from natural waters just prior to the spawning ground or by fish raised on farms.

## **TREATMENT OF STRIPPED EGGS ANND MILT**

Spawning male and female are removed from the holding tank and dried with a towel. The genital area is lightly pressed to remove faeces from the lower gut and any interstitial tissue which may contaminate the gonad product.

The eggs of ovulating females and ripe males are stripped into plastic bowls and mixed by swirling or stripping with a feather or plastic spoon. Stripping is carried out by applying moderate pressure on the flank of the spawner which results in the release of the gonad products. If heavy pressure is required or blood is exuded the fish should not be used as it is probably not ready for spawning. Distilled water is then added to the mixture of eggs and milt. This solution temporarily reduces the stickiness of the eggs and prolongs the fertilizing capacity of sperm.

## **INCUBATION AND HATCHING**

The fertilized eggs can be incubated in a large inverted bottle until the flat bottom cut off. This bottle is called a zougar jar. A slow flow of well aerated, 25°C water is introduced at the bottom of the jar sufficient to just keep the eggs afloat. The water is allowed to overflow over the top of the jar.

At this stage, no difference can be seen between fertilized and unfertilized eggs and all must be incubated. However, 6-7 hours after incubation, unfertilized eggs become white and opaque and discontinue development. Because these eggs are prone to fungal or bacterial infections, which may be transmitted to the healthy fertilized eggs, they must be removed, usually by siphoning with a rubber tube.

The fertilized eggs are retained in this jar until they hatch. The yolk sac hatchling are transferred into shallow rearing troughs and trays where they grow until their yolk sacs are absorbed.

## **CARE OF THE LARVAE**

After absorption of their yolk sac, hatchlings are better fed with pure cultures of phytoplankton for about 2 to 5 days, depending on the species. As the larvae get large they are fed with zooplankton organisms such as Artemia larvae. Larvae clarias can be on formulated diet containing 37-40% crude protein. The feed is powdered through sieves of mesh sizes 55-65m and fed to the larvae twice daily. Larvae grow rapidly to the fingerling stage and these can be transferred to fingerling ponds after 3-4 weeks.

## **PRACTICAL INDUCED BREEDING**

The most common technique employed to induce final maturation and ovulation in African catfish is to inject the female with hormones or pituitary gland materials. The required quantity of powdered acetone dried pituitary material or the required number of whole pituitaries are pulverised in a porcelain mortar, mixed with the required quantity of physiological salt solution (9g of common salt/liter of water). A syringe is filled with the suspension and the injection can be given.

**Note:** Fill the syringe, insert the needle and supply the syringe again into the mortar, when this is possible you can start injecting the fish. This procedure has to be followed always, as the needle often gets blocked if the pituitary material is not completely crushed and it is unpleasant for the fish and annoying for the operator to resolve this problem once the needle is inserted into the fish.

The most common method of administering the hormone solution is by intra-muscular into the dorsal muscle.

**Note:** Cover the head of the breeder with a wet towel in order to keep it quiet during injection. In general, fish keep still if their eyes are covered.

### **MATURATION PROCESS AND STRIPPING OF EGGS**

The process of final maturation (migration of the nucleus fusion of the yolk, breakdown of the germinal vesicle meiotic division) and ovulation (rupture of the follicles and accumulation of ripe eggs in the ovary cavity) cannot be stopped or reversed after administration of the correct hormone dosage. Once these processes start, the egg can be spawned or stripped.

**Note:** normally, the females are injected in the afternoon and are kept (separated from the males) in holding facilities. The holding facility can be a concrete basin inside a hatchery, a happa inside pond or even a simple plastic bucket or a half drum will do. Of a major importance is that the breeders can be caught easily the morning after injection to avoid spoilage of eggs.

The speed of the process is dependent upon water temperature, the higher the temperature, the quicker the eggs ovulate. The relationship between temperature and the time taken for eggs to ovulate is given below:

**The time taken between injection and stripping of female  
Catfish in relation to water temperature**

Water Temperature	Time between injection and stripping (Hours)
20	21
21	18
22	15.5
23	13.5
24	12
25	11
26	10
27	9
28	8
29	7.5
30	7

**Note:** Sometimes with fluctuating water temperature, and in particular with higher temperature during the day, it is difficult to establish the actual mean water temperature. This can result in eggs being stripped too early with consequently very low hatching rate (5-19%). Eggs which are stripped too early tend to have a tready consistency. It is always much safer to strip the eggs later rather than earlier. If you are too early you will lose all your eggs, if you are too late you will lose some eggs. The eggs ooze out very easily if stripped at the right time.

Stripping of the female spawners is carried out by gently pressing their abdomen from the pectoral fin towards the genital papilla. Ovulated eggs will flow out easily in a thick jet from the genital vent and are usually collected into a dry plastic container. The ovulated eggs are more or less transparent, flattened and 1g contained approximately 600 eggs. Under normal conditions a “ripe” female ovulates a quantity of eggs which equal 15.20% of her own body weight. If the fish is stripped too early the eggs come out with difficulty, whereas they have a “flushy” appearance if they are stripped too late.

The males of the African catfish cannot be stripped and consequently the sperm can only be obtained by sacrificing a male. The male is killed and the body surface thoroughly dried after which the testis is disserted and placed in a mortar or a teacup. The testis is rapidly cut into small pieces using a scissor and finally the milt is pressed out with a pestle or a teaspoon.

## **HYPOPHYSATION**

Hypophysation is presently the most commonly used technique for the propagation of fish. It is employed not only in propagation experiments, but also in the commercial production of million fish.

Induced ovulation and spawning achieved through hypophysation amount to a short cut of the natural process. In nature, ovulation in a fish is regulated and brought about by its own gonadotropic hormone produced and stored by the pituitary gland. The stored hormone is released into the blood when all the requisite conditions become favourable. But in the hypophysation technique, gonadotropic hormone extracted from the pituitary of some other fish (donor) is injected into the breeder and this brings about the final ovulation.

Like all other techniques, this technique too has its own limitations. Some of the sensitive fish such as the pike-perch cannot tolerate the treatment, while others may ovulate only irregularly. Then again, the breeders whose ovaries have not yet reached the adequately ripe stage fail to respond to hypophysation. It is a fundamental rule that hypophysation will be effective only when the eggs in the ovary have reached the resting or dormant phase after the completion of *vitellogenesis*. The eggs are then materially ready for further development to be triggered by gonadotropic.