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ADVANCES IN AFRICAN MUDFISH SEED PRODUCTION: A REVIEW

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Abstract

This paper reviews the historical ways of acquiring African mudfish seed for production. It discusses how fish seed were acquired from the wild. This is followed by the method of injecting hormones to stimulate fish reproduction in captivity. To provide sufficient number of seed to meet the need for commercial aquaculture, the method of hypophysation involving the use of artificial hormones to enhance gonadal development and spawning was adopted and popularized. Different workers have examined different ways of improving output by focusing on fecundity, nutrition, growth and health of the brood stock and seeds.

Keywords: *Artificial reproduction, Clarias gariepinus, Advances, Hypophysation, Seed, Broods.*

1. Introduction

In Fisheries, 'seed' is a technical term used usually in the plural sense to refer either collectively to the juvenile life stages of fish (hatchling, spawn, fry and fingerling), or specifically to fry (the stage after the yolk sac disappears and before the scales form). The leading Aquaculture fish species in Nigeria is the African mud fish (*Clarias gariepinus*) (Olumuji and Mustapha, 2012). Many researchers submit that the general acceptability of this fish is brought about by its eating qualities which are a function of its tasty flesh as well as firm muscles (Ayotunde *et al*, 2011; Okey *et al*., 2019; Sunday *et al*., 2021). The primary reason why this species is cultured everywhere in Nigeria is its hardy characteristics. It can survive outside its usual dwelling place for up to four days. It, therefore, means that farmers with poor experience in fish culture can succeed in keeping it alive for a long period of time. This fish species accounts for the fast growth of the Aquaculture industry in Nigeria. The assertion that Aquaculture is the fastest growing food production industry in Nigeria especially, and all over the world in general has been reported (FAO, 2000; Eriegha and Ekokotu Ada, 2018; Sunday and Ada 2021). The growth in the Aquaculture industry is attributable, in part, to improvements in the provision of fish seed. The success of any modern farmer to produce fish in commercial quantities depends on the availability of seed.

In the distant past, fish seed may have been captured with adult fish during fish hunting. Persons with conservative minds may have kept the juveniles to grow further before consumption. This may have given birth to Aquaculture, which is the farming or care of living organisms in a controlled, aquatic environments for human use. In the distant past, therefore, man captured fish seed from the wild and raised them.

In many places including Nigeria, Egypt and Italy, feral catfish fries are caught from the wild when they are making reproductive and trophic migrations. This is achieved by getting them directed into burrow pits and prevented from returning back to the main stream. In the pit confinement, they are allowed to grow to adult stages before being harvested.

The general techniques of capturing juveniles from the wild were, and are still, full of challenges. Persons distant from water bodies cannot practice this type of juvenile fish acquisition by burrow pit.

Other challenges include the inability of the farmer to obtain the seed in the desired quantities from the wild, and especially, at the time they are needed. This makes impossible for the farmer to plan his production cycle and renders him inactive some of the time. Dwindling wild stocks make it impossible for this kind of fingerlings sourcing to continue (Bisht *et al.*, 2013), hence farming activity cannot be programmable. Natural fish seed production in the wild is seasonal. Periods when the seed are not available for culture cannot provide income for those who rely on it for sustenance. Fish seed become available only during the onset of the rains in the tropics. It is, therefore, not possible for a farmer to procure the seed when needed. The period of the onset of rains is usually characterized by unfriendly environmental conditions such as fluctuating temperature, turbidity, conductivity, low dissolved oxygen levels and dissolved substances. Fry survival during such periods is low because of the unfavorable environmental conditions. The fries maybe infested and could be harvested along with parasites or the dormant stages of the parasites that will continue to develop in the fish. Reports of life cycles of human parasites using aquatic species of organisms as intermediate hosts abound in literature (Sunday and Ada, 2021; Keith, 2018; Fukumoto *et al.*, 1988). Natural environments do not have sufficient food. This leads to the stunting in the growth of early stages and consequent impaired development. The selection of specimens in this situation into fast growers and the stunts is difficult. These challenges caused fish farmers to look for alternative ways of propagating fish seed (Shukla *et al.*, 2021). Acquisition of fish seed from the wild has even worsened with the introduction of pesticides especially herbicides into water bodies. Records have it that the application of herbicides into natural water bodies is increasing (Cox, 2004). When herbicides are applied into the water, destroy fish habitats as well. Some ignorant fish hunters or fish harvesters apply obnoxious methods such as poisons (e.g., Gamalin 20) to kill fish in their natural environments and pick the dead bodies of fish. This method is known to kill not only the targets organisms only, but also other organisms including the juvenile stages that are too small to be used by the hunters.

Since herbicides, are able to destroy weeds on a large scale before or on emergence without interfering with the crops and with ability to reduce heavy dependence on human labor, their importance has been and will continue to be felt. Their rate of use is expected to increase in the tropics in the nearest future, especially as crop that are genetically modified to tolerate herbicides are continuing to emerge (Cox, 2004; Asogwa and Dongo, 2009). The residues of these chemicals including soaps and detergents end up in the aquatic ecosystems (Ogundele *et al.*, 2004). All these result in habitat destruction.

Ogunsina (2014) posited that African Catfish Hatchery came into existence due to the difficulties of getting consistent, fast growing, disease-resistant and uniform sized catfish fingerlings and juveniles from the wild.

These methods, which were likely developed stepwise, include confined reproduction, semi natural propagation and artificial breeding/hypophysation.

Natural spawning /Confined reproduction

Fish broods which were harvested, kept in captivity to reproduce must have had environmental conditions which approximated to those in the wild. The condition of light affects reproduction of organisms in two ways. These are light intensity and daily length of light availability (photoperiodism). Fish has been seen to spawn earlier than normal due to enhanced photoperiodism (Mustapha *et al*., 2012; Adebayo, 2018; Solomon and Okomoda).

Temperature is influential in the rate of development of organisms. Rate of metabolism is linked to temperature and directly affects the speed of reactions within the optimum limits outside which the metabolic enzymes are deactivated (Adebayo, 2018). Sapkale (2011) and Eriegha and Ekokotu (2017) observed that *Clarias gariepinus* could spawn successfully when the temperature is between 25 and 32^{0}C with optimum at 28 to 30^{0}C.

Chattopadhyay (2013) reported the spawning of fish based on contagion. This is described as sympathetic breeding. This is a situation where some members begin to spawn because they see others spawned. In Bundh breeding, this phenomenon helped to reduce the quantity of hormone used. This is because if some members are injected with hormones, they will spawn. Others

that are close by would spawn following the spawning of the injected members. This phenomenon is not only in fish. It is even observed in humans and is responsible for different ages in which different human populations begin to reproduce or marry.
Factors like rain water, which is fresh water in flooded conditions, can trigger spawning. This may be the reason for the movement of *Heterobranchus* upstream at the beginning of rains for spawning. Early rain rushes to the stream to dilute the water and lower electrolytes concentrations. This induces gonadal hydration resulting in spawning. This forms part of the explanation why many fish spawn immediately after heavy showers (FAO, 2021; Lucas, *et al.*,2001).

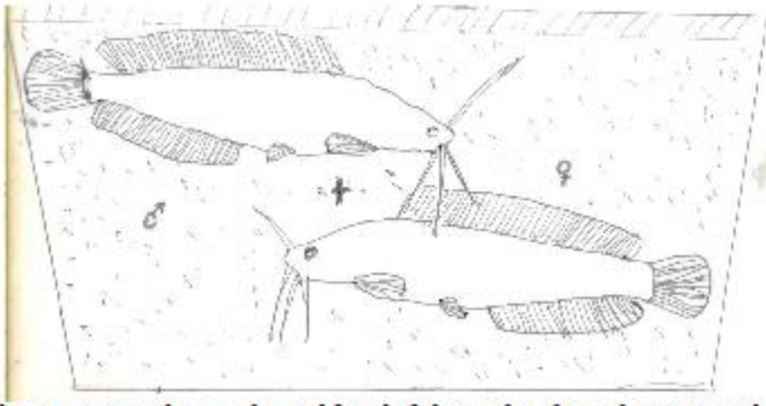
In this *Natural propagation***,** males and females are placed together in *a breeding area* such as a small pond or an enclosure where they spawn naturally.
The successful breeding of certain *Clarias* species may require some more *environmental manipulation* such as the inflow of new water and a sudden rise of the pond water level of water and the presence of grassy vegetation or the presence of artificial nests for American and European catfishes, *Ictalurus* and *Silurus (Yisa, et al.*,2013).

Semi natural propagation:
The fish (usually the females only) are first given *an injection of chemicals*, such as a *pituitary gland* extract, which will trigger spawning. Males and females are then placed together in a specially prepared breeding area such as a small grassy pond or an enclosure where spawning takes place. The *fertilized eggs* are usually collected and reared under improved conditions, either natural or artificial (Woynarovich *et al.*, 1980).

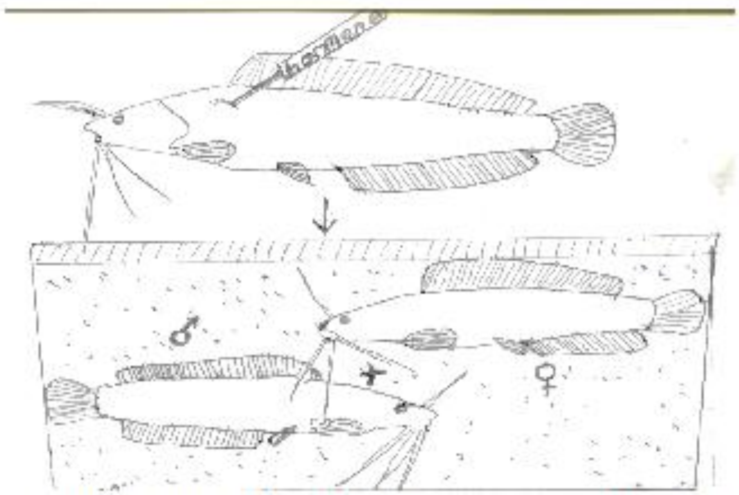
Artificial propagation: There are cases when many broods obtained from wild and reared in captivity with inappropriate environmental conditions. In such cases, reproductive development could be arrested in late vitello genesis stage. This is the reason for hormonal administration to stimulate gamete maturation (Zohar. *et al*., 2001). According to Shakla *et al* (2021), seed production, apart from wild capture started in the 16th Century AD. Ecological method in which a simulation of what happens in nature was carried out in China 2,400 years ago by Fan Li. It is in Argentina that Houssay (1930) started induced breeding by applying pituitary hormone to spawn fish. In 1935, Von Inherring initiated hypophysation of a viviparous catfish in Brazil.

Von Inherring and many other workers succeeded in artificial breeding of carp using induced breeding in the 1950s. Indians; Ramaswami (1956) and Sundararaja (1957) were the first to record success in spawning of catfishes after hormonal injections. The females are given one or *more injections of chemicals* which regulate the final ripening of *dormant eggs* in the ovaries. As soon as the eggs are ripe, they are stripped from the females. Eggs are *artificially fertilized* with sperm obtained from the males and reared under *controlled conditions* (Woynarovich *et al.*,1980).
Success in artificial production of fish seeds is hinged on several basic factors. These include; obtaining viable broods, the ability to manipulate the gametes from the fish, hatching of viable fry, feeding the fry with appropriate diets and maintaining the fry in environment favorable for their fast growth and survival and simplification of the process and passing it to local farmers.

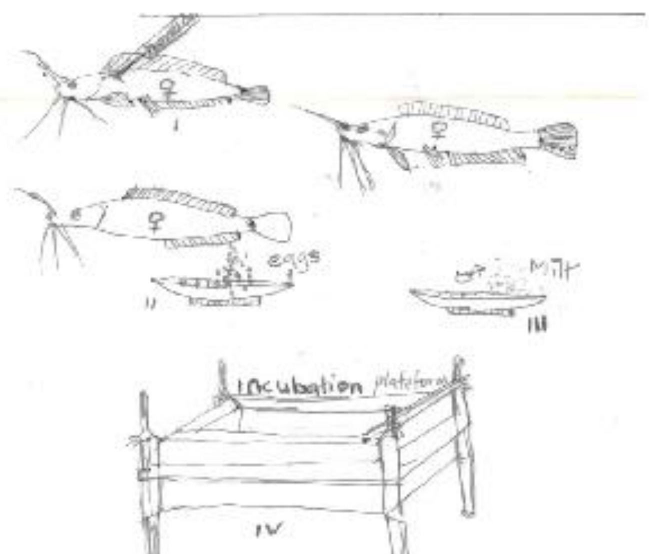
Source and care for broods: Broods could be captured from the wild. They need to be handled with care during transportation. Care during transportation can be enhanced by using tranquilizers (Okey, 2019). They need to be disinfected and de-parasitized with a formaldehyde bath of 15 ppm for 6 hours. Bacteria can be removed by applying Furaltadone or Furazolidone, at 10 ppm for 1 hour together with a fungicide (Malachite green 0.2 pm) daily to prevent outbreak of infections, due to stress during transportation.
Due to scarcity of broods, one may decide to rear fingerlings up to become breeders in the farm. This is better because jumpers (those that grow very fast) can be selected to become broods. It was found that brood-stock maintained for at least one year under controlled conditions in a hatchery will lose its seasonal reproductive cycle. This would mean that mature breeders will be available for year-round production (Legendre, 1986).
Individual brood fish of weighing 0.5–1.0 kg is acceptable. They have a substantial quantity of mature eggs, but larger fish have been reported to



Natural propagation where males and female fish are placed together in reproductive tank



Semi natural propagation where the female is given hormonal injection to prepare it for spanning, before the male and female are placed together in a reproductive tank



In artificial propercation the female is given injections to quicken the ripening of eggs (1). The eggs are stripped into a plastic plate (ii) and in (iii), the milt is obtained by murdering the male or by operation the sperm and eggs are mixed (fertilization) before being placed in cacaban for incubation (iv) have larger and more viable eggs (Bichi,2015). It has been found that the same female brood fish can be induced to reproduce artificially up to 8 – 10 times a year with no harm to broods or eggs (Legendre, 1986).

Optimal stocking density of brood is 100 – 150 Kg/m^2. Dissolved oxygen content of the water should not fall below 3 ppm. The optimum temperature recommended for rearing and conditioning brood fish for artificial propagation is 25°C (Richter *et al*, 1982). The optimum temperature with a minimum of fluctuation is a prerequisite for quantitatively and qualitatively adequate gonadal development all-year round. Illuminated environment may irritate catfish since their preferred habitats are turbid waters. Little or no disturbance should be around breeding facility as disturbances interfere with normal gonadal development (Woynarovich and Horvath, 1980).

For a grow-out fish, feeding level is usually 5 – 6 % of the body weight (Sunday et al., 2021), 1 % is recommended for brood fish of 500 g or more divided into about 4 rations to avoid contamination (Richter *et al,* 1982). In Selection of brood fish for artificial propagation, the females which show the most advanced maturity are chosen using the following criteria: well-rounded and soft abdomen which extends anterior past the pectoral fins to the urogenital papilla;mature eggs, showing clearly the nucleus in the centre, can be obtained easily by slight pressure on the abdomen; and the genital opening is swollen and sometimes reddish or rose in color. There are no clear external symptoms to indicate the maturity of the males. Some authors describe a more elongated slightly swollen urogenital papilla for "ready-to spawn" males.

In practice, it is convenient to select the breeders in the morning and to inject the hormone solution in the evening. Breeders are kept separately according to sexes in well-oxygenated, clean water and covered with a board to avoid escaping. Careful handling, using a hand net and wet towel is necessary to guarantee proper health condition of the breeders. The selected breeders are not fed between their selection for reproduction and stripping.

The ability to manipulate the gametes from the fish: The breeding of catfish in hatcheries requires that male be killed. This usually causes shortage of male broods. It is also observed that on many occasions, after killing the males in search of milt; the gonads are devoid of milt. Experience has shown that this is possible even after the papilla has shown redness, which is a sign pointing to their maturity (Idahor *et al.*, 2018). It has been reported that males cannot be stripped of their milt without killing them even after hormonal injections (Diyaware *et al.*, 2010; Korede and Odedeyi, 2017). The reason for failure to strip the sperms is not that they have failed to produce sperms, but it is because the seminal vesicle blocks the urethra while trying to strip the male fish. Korede and Odedeyi (2017) also agreed that the position of the testes which is placed dorsal to the intestine makes it difficult to exert pressure on the testes through the gut. That means that pressure exerted on the abdomen is being absorbed by the digestive system. Yisa (2013) demonstrated that over dose of ovaprim (1.25 – 1.5 mg of ovaprim/Kg of male fish) can result in successful hand stripping in which; the fish can recover with appropriate feeding. In 2019, Promina and Petrushin devised another means through which milt can be obtained from the males of African catfish (*Clarias gariepinus*) and European catfish (*Slurus glanis*) without sacrificing the males.

Apart from sacrificing the male fish, dissection to remove the testes often leads to a problem of the milt being contaminated by urine. This quickly leads to sperm activation and death before they are needed (Viveiros, 2003). To avoid this, Promina and Petrushin (2019) established and recommended the use of catheterization of urinary bladder (insertion of catheter in the urinary bladder to remove urine) and then the collection of milt by partial gonadosectomy. The advantages of this method include the fact that: it is conservative in approach; the testes can regenerate; all testes can be removed and the fish is fattened for sell at a later time. All these advantages are leading to closing the gap of male brood shortage.

The total amount of milt that can be produced and collected from one male of *Clarias gariepinus* is a maximum of 5 ml (Okunsebor *et al* 2016). This is a problem because this volume of milt will not go a long way to fertilize a large number of eggs. Apart from the small volume produced, the milt is sticky and may not live long. Akanmu *et al*. (2019) used extender, coconut (*Cocos nucifera*) water to extend the life and activities of the sperm. In nature, *Cocos nucifera* water has been used as a physiological solution. It is reported that it was been used for rehydration during the World War II. (Weimar, 2011). Akanmu *et al*. (2019) obtained similar results with *Cocos nucifera* water in the extension of the life of *Clarias gariepinus* milt with those achieved with saline solution. While saline solution has only sodium chloride as a solute, coconut water has a large range of nutrients including vitamins (Akanmu *et al*., 2019). If it is for the purpose of fish fertilization only, then saline solution should be used due to its low cost and availability compared to coconut water, yet the two produce similar results. A saline solution is a mixture of 9 g of common salt (NaCl) in 1 litre of water. Ukwuani *et al*. (2014) estimated that 0.6 ml /g of milt is optimum for use. These workers reported that 1 g of milt after mixing with saline water can fertilize 10 g of eggs

To prepare saline solution, A = 8 g of NaCl + 1 L of H_2O = A
To dilute milt B for use = 0.6 ml of saline water + 1 g of milt = B
To fertilize each 10 g of egg =1 g of B + 10 g.
To fertilize y weight of eggs, we need 1/10. y quantity of milt.

0.6 ml of saline water + 1 g of milt to fertilize 10 g of eggs could produce the maximum rate of fertilization and survival in hatchling of *Clarias gariepinus*. It is known that salinity at extremes is not favorable for the survival of cells. At optimum concentration, saline solution creates a medium for sperm motility and larger surface area for the sperms during fertilization of the eggs.

Sourcing safe hormones and administration: Hormones are said to take about 50 per cent of the cost of fingerlings production. To source for cheap alternative for hormones, several workers have even researched on non-piscine organisms to obtain reproduction stimulating hormones. Workers like Fagbenro *et al.* (1993) and Salami *et al* (1993) have used the pituitary of anurans (tailless amphibians) to get cheaper hormones.

Induction of reproduction involves the use of natural or synthetic hormones. Mosha (2018) reviewed several papers especially from West Africa and came up with a conclusion that both synthetic and non-synthetic hormones are capable of ensuring availability of quality sperms and eggs. Mosha (2018) compared natural and artificial hormones in the areas of their effects on fish's spawning ability, quality of sperms, number of eggs produced, fertilization, hatchability, survival rate and growth of larvae. In males, Korede and Odedeyi (2017) compared milt volume, number of spermatozoa in milt, sperm concentration, sperm mobility and duration of mobility. Mosha (2018) and Oyeleye *et al.* (2016) however recommended ovaprim from ovatide, ovulin, pituitary gland extract, ovaryprim, ovopel, dagin and aquaspawn as the best hormone for the production of *Clarias gariepinus*. That means, Ovaprim, a product of Drs Lin (a Chinese) and Peter (a Canadian) is preferred over others. Ovaprim is sold as a ready-for-use product. Due to the high cost, Olumuji. and Mustapha (2012) tested its potency after serial dilution with saline solution but discovered that it lost its potency beyond 50 per cent dilution. Dilution was also found to extend the latency period.

Achionye-Nzeh and Obaroh (2012) reported that the number of eggs released increased with increasing concentration of ovaprim up to 1.5mg/Kg. Such high concentration (1.5 mg/Kg) reduced the latency period to less than 12 hours and also increased the fecundity of the fish (Sharma *et al*., 2010; Achionye-Nzeh and Obaroh, 2012). For the health of the female fish, higher concentrations (above 0.5 mg/Kg) favor the ease with which the eggs ooze out to avoid over pressing the abdomen. Too much pressure application to the fish's stomach can lead to post spawning stress and mortality (Achionye-Nzeh and Obaroh, 2012).

However, due to the natural availability of natural hormones within the farm, which are much cheaper than their imported synthetic counterparts, the natural hormones could be used. When natural hormones present in the hypophysis are used, the process is described as *hypophysation*. Ovaprim is preferred over natural hormones because Deoxycorticosterone Acetate (DOCA) causes severe ulcer on the female injected fish; human chorionic gonadotropin (HCG) is expensive and the fish pituitary extract (PGE) is difficult to quantify.

Embryology: Knowledge of embryonic development is important in successful seed production because certain factors influence that the process have to be known. Work on the embryology of *Clarias gariepinus* has been carried out by Legendre and Teugel (1991) and Haylor and Mollah (1995). After the union of male and female gametes, a zygote is formed. Olaniyi and Omitogun (2013) studied the development from zygote to early stages of the fry of *Clarias gariepinus*. It is at these embryonal stages that some genetic manipulations can be achieved (Agnese and Teugels, 2001).

Fry feeding and environmental maintenance: Kristant, *et al*. (1998) reported that incubation method did not affect the hatching rate and survival of the silu rid fish (*Pangasius hypothalamus*) within four days. They pointed that what could affect hatching and survival was moderate agitation of eggs.

Summary and conclusion

The production of fish is a biological process. That means fish production must start from the reproduction of stocking(planting) material. Reproduction is a natural process. It is the reason why hunters have been able to harvest both adults and fish seed from the natural environment. In the distant past, the aim of hunters was to get food for immediate consumption. Like Agriculture, Aquaculture may have started when hunters(fishers) were able to preserve a portion of their catch which was more than their need for immediate consumption for future use. The practice of culture (preservation) may have become elaborate when the hunters were able to preserve the juvenile portion of their catch and grow them to table size.

As the need to increase fish production grew, harvesting of fish seed for raising in confined environments increased correspondingly. The increase in fish capture for both consumption and cultivation led to the depletion of the wild stock. The need to look for fish seed by manipulating the fish to reproduce outside the natural environment arose. Some fishes are able to reproduce outside their natural environment. Most siluriformes (catfishes) find it difficult to reproduce outside their natural environment. Studies were done to know what may have been responsible for their failure to reproduce in artificial environments. It was discovered that factors like salinity, temperature, pH, conductivity, state of health and nutrition contribute to their reproduction success.

A situation where the fish were left in to reproduce on their own in an artificial environment is called artificial reproduction. A situation where reproduction is initiated by injecting the fish with chemicals (hormones), is described as semi natural reproduction. This came into play when some researchers became aware that these hormones were useful in stimulating gonadal development. An Argentine, Hossey who won the Nobel prize in Physiology was the first to start hypophysation. This is the practice of using the hypophyses, which contain reproductive hormones to inject the fish so that reproduction could be kickstarted.

Since the discovery of Hossey, a lot of innovations have been carried out to improve artificial reproduction in fish. Several synthetic hormones have been produced. Environmental factors which favor healthy growth of the early stages of fish have been identified. Work is still on-going to study other factors that could improve artificial reproduction in the clariids. While a lot of production of the clariid catfish, *Clarias gariepinus* has been achieved and is still being achieved, not much has been done for its relative, *Heterobranchus* which is more difficult to manipulate to reproduce artificially.

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