A short review on induced spawning and seed production of African Catfish Clarias gariepinus in Malaysia

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Abstract. African catfish Clarias gariepinus is one among the highly prized freshwater fish species. The most important criteria for successful propagation of any cultivable fish species in aquaculture is the availability of sufficient quantity larvae of uniform size, quality, and free of diseases, parasites, and pests at the time of stocking in culture ponds. Traditional methods of induced spawning for fish are based on the injection of gonadotropic hormones from different sources, including extract of carp pituitary gland, partially purified fish gonadotropic hormones and mammalian gonadotropic hormones, especially human chorionic gonadotropin (HCG). Several synthetic analogues of gonadotropin-releasing hormones (GnRH) and preparations have been used in fish breeding and seed production in different fish species. GnRH and its super-active analogues have been used in catfish breeding. Such treatment results in the release of the fish own gonadotropin. The use of natural and synthetic hormones in the induction of spawning and seed production in air-breathing fishes is well documented. This paper will review the research conducted on induced spawning and seed production of African catfish (Clarias gariepinus) in Malaysia.

1. Introduction

The world aquaculture has been growing strongly in many countries, mainly due to the advances in the technical management of fish cultivation to cope with the increasing worldwide demand for fish as well as the economic and environmental sustainability of fish farming. During the past decade, the culture of air-breathing fish species has been increased dramatically and is now a significant global source of protein for human consumption. Several species of air-breathing fish species are now farmed extensively including snakehead fishes Channa spp., striped catfish Pangasianodon hypophthalmus, pangas catfish, Pangasius pangasius, catfishes; Clarias spp., and swamp eels; Monopterus spp., etc.

The single most important drawback of the large-scale commercial culture of several fish species especially air-breathing fish species is the deficiency of quality seed of uniform size, and free of diseases, parasites, and pests at the time of stocking in culture ponds. These strict conditions are seldom fulfilled when the fish seeds are collected from natural water bodies [1]. Furthermore, the brood fish that are obtained from the wild condition and brought to captivity and reared in captive conditions may receive inappropriate environmental cues for reproduction and these can cause reproductive development to be arrested in late vitellogenesis stage. For this reason,
hormonal administration has been warranted for stimulating of gametes maturation and been successfully used to spawn many fish species that exhibit arrested reproductive development [1]. The important organs and glands involved in this hormonal cascade are the hypothalamus, pituitary gland, and gonads (Fig. 1). It is well known that reproductive processes in fishes are controlled by endogenous biological rhythms as well as by environmental cues [2]. Endogenous control is mediated through actions of various hormones along the brain-hypothalamus-pituitary-gonad axis. Under natural conditions environmental stimuli are detected and relayed to the brain, resulting in a release of hormones and neurotransmitters that regulate ovulation [3, 4]. The most important reproductive hormone is gonadotropin-releasing hormone (GnRH) that regulates gonadotropic hormone, GtH [4]. Gonadotropin release in teleost fishes is also influenced by a gonadotropin-inhibiting factor (GRIF) from the hypothalamus. This factor has been identified as dopamine and demonstrated to have inhibitory activity on the release of GtH [5].

2. Induced Spawning
The developments of induced spawning techniques have allowed farmers to profitably breed and culture fish species that do not naturally reproduce under captive conditions. Over the last few decades, hormonal administration techniques have been used to induce final oocyte maturation and spawning in fishes which allowed the reproduction in controlled conditions [6-8]. Moreover, induced breeding techniques have significantly contributed a lot to the expansion and diversification of the aquaculture industry [1]. The injection of different spawning agents in fish was adopted for successful ovulation and collection of eggs. Traditional methods of induced spawning in fish are based on the injection of GtH-II from different sources, including extract of carp pituitary gland, partially purified fish GtH-II and mammalian GtH, especially human chorionic gonadotropin (HCG) [9-12]. The GnRHa and domperidone are the most popular compounds for induction of ovulation and spermiation in various fish species. The introduction of GnRH analogues has been proven to be efficient in inducing maturation and spawning in many fish species [13-20]. Similarly, an antidopaminergic drug, pimozide has also been found to be highly effective for stimulating the spawning process of fishes mainly in cyprinids and catfishes [21, 22]. These hormones are used to stimulate the secretion of endogenous gonadotropin [23].

![Figure 1. The natural hormonal cascade of events in natural and induced spawning](image-url)

Hormone administration is the most common method of induced breeding in fishes which the pituitary extract injected into the matured male and female fishes and the hormone induce them to
spawn. Successful induced spawning depends upon the dosage of hormone injection, the stage of maturity of the fishes and environmental condition such as temperature, water currents, and rain. Lin and Peter [24] reported that among the several inducing agents used in fish breeding, salmon gonadotropin releasing hormone (sGnRH) or luteinising hormone releasing hormone (LHRH) analogues in combination with dopamine antagonists were identified as an effective agent in fish breeding and seed production. Several practical problems have been reported using exogenous hormones and endogenous hormones, such as weighing of such low quantity, preparation of these analogues and storage of this hormone in prepared solutions. Due to these difficulties, fish breeders and farmers are unwilling to use these hormonal preparations under farm conditions. Several commercially available synthetic ovulating agents in a ready-made form which contained GnRHα and dopamine antagonist such as ovaprim, ovatide, ovopel, dafin and aquaspawn are becoming very popular nowadays and found to be efficient and successful spawning agents in different fish species [25-28]. Successful spawning through a synthetic analogue of GnRH has been reported in several air-breathing fish species including *Clarias batrachus* [29 -30] *Heteropneustes fossilis* [31-33], snakehead fish [6,7, 34]. This paper will review the research conducted on induced spawning and seed production of African catfish (*Clarias gariepinus*).

3. Breeding

African catfish, *Clarias gariepinus* is one among the highly demanded freshwater food fish and cultivar species in Malaysia and other Asian and African countries due to its higher resistance to diseases, ability to tolerate a wide range of environmental parameters and high stocking culture conditions, relatively fast growth rate and good quality meat [35-39]. It is one of the popular native fish species of African countries and has been introduced and commercially cultured in several countries in Europe (Netherlands, Germany, and Belgium) and Asian countries (Indonesia, Thailand and Malaysia) and South America (Brazil). *C. gariepinus* inhabits a wide range of water bodies like swamps, lakes, and rivers. It is a hardy fish and is able to thrive in harsh environmental conditions such as muddy, turbid and oxygen depleted water bodies with the help of accessory air-breathing organ which allows them to breathe oxygen from the atmospheric air. Generally, *C. gariepinus* is omnivorous in nature and usually feeds on insects, plankton, snails and plant matters in the natural water bodies [40, 41]. However, this species is highly cannibalistic when substantial differences occur in size. African catfish breeds naturally during the rainy season in flooded rivers, inundated paddy fields, and earthen ponds. The seed collection of this species from the wild is unreliable, time-consuming and uneconomical for large-scale culture of this fish. To overcome these problems, induced spawning is thought to be the only alternative method for seed production and supply. Marimuthu et al. [42, 43] reported the induced spawning and seed production of African catfish.

Proper selection of brood fish is the key to the success of induced breeding and seed production. The selected brood fishes should be healthy and fully ripe. Brood fishes can be collected from their natural habitat quite in advance of their breeding season and raised in fertilization pond of the fish farm. The two sexes can easily be distinguished by the shape of the belly and by the form of the genital papilla. Unlike other freshwater fish like carps, milt cannot be squeezed from the males. Milt from two males with body weight 2 kg can fertilize the eggs of ten females of similar age and size. Final maturation of eggs and sperm and release of eggs from the female ovary are induced by injection of both males and females with an appropriate dosage of spawning agents. Several inducing agents and dosages have been studied and reported. Fish are removed from the holding tank and the body weight is taken for calculation of hormone dose. For hormone injection and handling, the fishes can be immobilized by the partial wrapping of the body in soft-textured cloth or tissue paper. The hormone is administered intramuscularly below the anterior part of the
dorsal fin and just above the lateral line. Immediately after hormone administration, the male and female fishes are separately released into the cement or circular plastic tanks at 28–30ºC.

Table 1. Induced spawning of African catfish, *Clarias gariepinus* using synthetic hormone ovaprim (Adopted from Marimuthu et al. [42])

| Hormone dose mL/kg BW | Female fish weight (kg) | Male fish weight (kg) | Total egg mass (g) | Fertilization rate (%) | Incubation period (h) | Hatching (%) |
|-----------------------|------------------------|-----------------------|-------------------|------------------------|-----------------------|-------------|
| 0.4                   | 1.98 ± 0.73            | 2.53 ± 0.46           | 300 ± 90.0        | 97.88 ± 0.38           | 27.6 ± 1.2           | 93.66 ± 2.96 |
| 0.5                   | 1.83 ± 0.15            | 2.80 ± 0.10           | 167 ± 61.0        | 92.77 ± 5.59           | 23.5 ± 1.0           | 85.77 ± 7.47 |
| 0.6                   | 1.46 ± 0.46            | 2.46 ± 0.15           | 163 ± 60.0        | 95.66 ± 3.67           | 24.0 ± 0.9           | 83.66 ± 2.51 |

The brood fish holding tanks should be covered with plastic netting. Ten to twelve hours after hormone injection, the male and female fishes are carefully taken from the tank for egg and sperm collection. The body of the fish is dried with paper towels or cotton cloth; the water should not drip into and mix with the egg mass while stripping the eggs. Eggs are stripped manually from females by applying slight pressure on the abdomen towards the genital pore. The eggs are collected in a dry circular plastic container. The testes are surgically removed and placed in a petri dish. Testes tissues are cut or chopped into small pieces using a scalpel or single edged razor blade, and mixed with about 5-10 ml water and then macerated to obtain a sperm suspension. The suspension is then added to the eggs immediately upon preparation and evenly mixed for fertilization. The egg mass with sperm is swirled gently to ensure proper mixing. After 10–15 seconds, water is added to cover the eggs to stimulate the motility of the sperm and fertilize the eggs. After five minutes the eggs are washed thoroughly with clean chlorine-free aged tap water. During this time any debris and tissue particles need to be removed from the mixture. Marimuthu et al. [42] reported the successful induced spawning and seed production of African catfish using ovaprim and the results given in Table 1. The results of the study shown that, the synthetic gonadotropin-releasing hormone with a dopamine antagonist at the low dose of 0.4 mL/kg BW could be used as an appropriate spawning agent for successful breeding and seed production of *C. gariepinus*. African catfish produce the adhesive eggs causes the eggs sticking each other and resulted low fertilization and hatching rates. Several materials have been tested to remove the adhesiveness of the eggs, for example urea, mud, milk and colin [43]. In addition, the support the breeding program of this catfish, the cryopreservation method of this species has been well developed.

4. Conclusion
African catfish *Clarias gariepinus* has good market potential Malaysia. It also fetches a relatively high market price. The demand for seed has increased steadily and several farmers have engaged in catfish culture. The technologies for breeding and seed production for this species has been developed and the techniques can be easily adopted by fish farmers. The growth and survival of larvae and fingerlings depend on the careful management of rearing tanks, feeding and size grading. It has been felt that snakeheads and African catfish culture depend solely on the supply of quality seed and careful management throughout the larval rearing phase. More concerted effort and care need to be provided during incubation, rearing of early larvae, fry and fingerling stages for successful rearing and profitable catfish aquaculture.
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