Development of Genetically Improved Farmed African Catfish, *Clarias gariepinus*; A Review and Lessons Learned from Indonesian Fish Breeding Program

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Abstract. African catfish, *Clarias gariepinus*, is an introduced species and has been an important food supporting the fulfillment of food security in Indonesia. Along with increasing popularity as a farmed fish, it gradually showed lower aquaculture performance due in part to the deterioration of genetic quality of fry resulting from inappropriate and uncontrolled of broodstock. An African catfish breeding program aimed to cope with the problem was set in 2010 at the Research Institute for Fish Breeding, Sukamandi, Indonesia. Started with four founder populations, namely Egypt (29%), Paiton (27%), Sangkuriang (24%), and Dumbo (20%), a synthetic base population was established in 2011, and an individual selection targeting on growth improvement was conducted. A hundred broodstock were spawned to produce breeding candidates, and 5 percent of best-performing fish were selected in each generation. Genetic parameters (genetic gain and genetic variability) and a series of aquaculture performance-related traits including growth, feed conversion ratio (FCR), productivity, resistance to disease, size uniformity, and benefit/cost ratio were recorded. Following three successive generations of selection, over 50% of the accumulative genetic gain was obtained. This genetic gain consisted of 20%, 11%, and 20% from the first, second, and third generations, respectively. Field farm tests aiming at comparing the aquaculture performance of the selected strain against the existed local strains also showed promising results. It was 10-40% better in growth, 15-70% better in productivity, 2-9 times higher in benefit-cost ratio, shorter growing period (45-60 days), lower feed conversion ratio (0.6-0.8 in nursery and 0.6-1.0 in grow out) and higher survival (60-70%) following challenge test against *Aeromonas hydrophyla* infection. It also produced a higher uniformity in size, 80-90% in the fry production stage, and 70-80% in grow-out production. It seemed that there was an indication of positively correlated responses among growth and those traits. Selective breeding in African catfish by applying individual selection carried out at RIFB was managed to obtain a significant genetic improvement while maintaining genetic diversity.

1. Introduction

African catfish, *Clarias gariepinus*, has been an important freshwater farmed species in Indonesia. Its production reached nearly 400 thousand metric tons in 2015[1], the second biggest after tilapia. The popularity of African catfish as farmed species in Indonesia dated back in 1985 when this species was introduced to Indonesia. It has superior farming performance relative to the local species, *Clarias batrachus*. Additionally, it is relatively easy to reproduce that most seed-producing farmers can produce seed through either natural or induced spawning techniques. While its simple reproduction
has been advantageous for providing the quantity of seed, it tends to gradually deteriorate genetic quality due to a lack of understanding of most seed-producing farmers on proper broodstock management. It has been indicated by, for instance, a declined in pond production, high size variation at harvest, and prone to diseases. To address the problem breeding program aiming at improving the productivity of African catfish was set at the Research Institute for Fish Breeding (RIFB) in 2010.

Following five years of research and development (2010-2015), a new strain of African catfish, named Mutiara resulting from a breeding program carried out by RIFB, was officially launched in 2015 [2]. Although the major trait to improve was growth performance, several other economically important traits were also getting improved. Due to these advantages, the strain is in high demand. To date, the strain has been disseminated and is used by farmers in 29 of 34 provinces in Indonesia. It has been one of the most successful products resulting from a fish breeding program.

Partial reports related to this breeding program have been published. However, these reports were scattered in various publications, and each of them touched on a specific aspect. No comprehensive report has been made available, making it difficult to do an evaluation and to take some lessons, both successes and failures of this breeding program. This review paper is the first to compile the milestones of the program and, at the same time, to evaluate various critical components of selective breeding of African catfish in Indonesia. Given the broad aspect involved in a breeding program, we limit the review by focusing on several key issues. They are 1) establishment of the base population, 2) the implementation of individual selection to improve growth performance, (3) Field testing of products in farmer level, (4) Putative correlated response to selection on growth performance, and (5) dissemination the results of the breeding program.

The objectives of the present report are to provide a holistic picture of a selective breeding program carried out to improve growth performance applying individual selection in African catfish, and to take valuable lessons that may improve the practice of breeding program, which may apply not only to this species but also to other economically important fish species. To be able to take lessons from this breeding program, it is a prerequisite to have a full understanding of the whole process. The structure of each section of this report is presented in a way that will facilitate readers to achieve that goal. It is introduced with brief information related to the topic of the section, the implemented procedure, the result obtained, and finally, it is closed with some notes on the lessons that can be drawn from the topic discussed in the section.

2. Founder Populations and the Establishment of a Base Population

African catfish is a non-native species in Indonesia. It was introduced in 1985 from Taiwan [3]. It showed a better farming performance and was able to attain a much bigger size (jumbo) than the local species, Clarias batrachus. Due to this characteristic, it has gained much popularity among farmers. They then recognized and named this population as Dumbo strain. A series of introduction of African catfish, both imported directly from the African countries of origin such as Kenya and Egypt and through the non-origin countries (Dutch and Thailand), proceeded following the first introduction. Among these populations, Egypt and Kenya were the latest introductions. The identity and names of these introduced populations in Indonesia were then known as strains of Dutch, Paiton, Egypt, Masamo, Sangkuriang, and Kenya [4].

Four populations, namely Dumbo, Sangkuriang, Paiton, and Egypt, were collected in 2010, and a series of molecular and farming performance test was carried out [5]. Molecular characterization using microsatellite genetic markers found that most of the genetic variation (95%) occurred within the population, while genetic differentiation among populations accounts for only 5% [6].

Based on the available stocks that RIFB researchers managed to collect, a preliminary farming performance evaluation [5], and molecular characterization using microsatellite markers [6], four populations were used as the founder to form the base population. These founder populations included Dumbo, Paiton, Sangkuriang, and Egypt strains. A total of 64 pairs of broodstock representing the four populations were mated in diallel crossing through artificial spawning carried out within the same day. While male broodstocks of each population contributed equally (25%) to the base population, the
The differential proportion was contributed by female broodstock founders. The highest contributing population (34%) was Egypt, followed by Paiton (27%) Sangkuriang (24%) and Dumbo (15%) [4]. The differential contribution among female populations was due to the failure of several females of particular population to spawn during artificial spawning.

The synthetic base population that was formed by applying those protocol turned out to have high variation, as measured by a coefficient of variation (CV), in growth performance at the larval stage (57.26%), nursery (52.84%), and grow out (69.01%). Additionally, the population also showed high growth performance, as measured by the specific growth rate (SGR) of 4.67%/day. These figures may suggest that the base population has a high degree of genetic variation and was an excellent raw material and starting point for a breeding program targeting on improvement of growth performance. Ten percent of the best performing individuals were then selected to be parents for the next generation. As shown by the selection response obtained in three successive generations of selection described below, the base population that was established seemed to have excellent genetic properties allowing the population to respond in a positive direction.

Successful breeding programs elsewhere, such as Atlantic salmon, rainbow trout, and GIFT tilapia, were also started with a composite, synthetic base population [7]. For instance, the base population of the GIFT strain of Nile tilapia was composed of 8 populations [8]. In comparison, the breeding program of Atlantic salmon was started with four founder populations coming from 41 river populations [9]. In simulated studies, [10, 11] found that the number of founder populations and mating strategy may affect the potential of genetic gain to be generated. They found that at least four founder populations, combined with mating of individuals within a population in the base population followed by mating across populations in subsequent generations, would result in a most significant genetic gain. Incorporating more number of founder populations would increase the potential genetic gain, but the increase is not substantial.

The breeding program of African catfish at RIFB was designed by taking into account the abovementioned information. By the time of implementation, four African catfish populations were managed to collect, namely Dumbo, Paiton, Sangkuriang, and Egypt. They were all mated to form the base population. The protocol implemented in establishing the base population adopted in this breeding, as described previously, seems to have been successful in providing genetic variation, allowing positive response to selections were generated throughout the breeding program.

3. Individual Selection for Growth improvement

Genetic improvement on economically important traits through selective breeding is allowed when heritable genetic variation exists on those traits. Previous studies found that growth performance in African catfish was potentially genetically controlled [12], that selective breeding could result in useful genetic improvement. Taking into account the ability to fully control species reproduction, the availability of grow-out facilities, and human resources, a simple method of a breeding program by means of individual selection was implemented [13].

Fifty pairs of selected broodstock from the base population were spawned to produce a population of breeding candidates for the next generation (G1). Mingled sperm from all males were used to fertilize 100 g eggs of individual female separately and were stocked in 1m2 hapas until hatching. The larvae were then raised in aquaria for 25 days, followed by rearing in a nursery for 30 days. The juvenile harvested from the nursery was then raised in a concrete grow-out pond for 60 days, at which time the selection of individuals to be broodstock for the next generation was carried out. The selection was conducted by taking 5 percent of the best performing individuals. Individuals of population average were also sampled for representing the control population. The selection response was calculated by comparing the performance of offspring derived from the selected and the control populations. A detailed description of technical aspects concerning the management of spawning, larval rearing, and nursery, as well as grow out, can be found in Iswanto et al. [13]. A similar sequence of activities was repeated for selection in the second and third generations [14, 15].
Following three generations of individual selection, over 50% cumulative response in growth performance was obtained (Table 1). This consisted of 20.59% in the first generation [13], 11.8% in the second generation, [14] and 20.24% in the third generation [15]. On average, 14% of genetic gain in growth performance was obtained in each generation of selection. The continuous and consistent genetic gain over three generations of selections is well expected. Being initiated with a broad genetic base in the base population, a genetic gain ranging from 11.8% to 20.6% within three generations, is within the range of those normally obtained by breeding program of other species, such as 10-15% in GIFT tilapia over six generations [16], and 8-10% per generation in salmon [17]. It is also expected that continuous genetic gain could be obtained when the selected program is continued for the next one or two generations. [18] found that for short term breeding program, namely up to maximum five generations, implementing selection based on individual performance by keeping 5% best performing individuals as broodstock for the next generation would result in a maximum response. However, the response may be reduced in a longer-term individual selection due in part to the accumulated inbreeding. Therefore, in order for the breeding program to keep generating genetic gain in the long run, different strategies have to be implemented. [18] has shown that selection based on best linear unbiased prediction (BLUP) could be the best alternative.

Table 1. Timeline and summary of core activities and results of the breeding program to improve growth performance through individual selection in African catfish, *Clarias gariepinus* at the Research Institute for Fish Breeding.

| Year  | Activity                                      | Selection Response (%) | Real Heritability | Reference |
|-------|-----------------------------------------------|------------------------|-------------------|-----------|
| 2010  | Collection of founders                        | -                      | -                 | -         |
| 2011  | Establishment of a base population            | -                      | -                 | -         |
| 2012  | Selection generation-1                        | 20.59                  | 20.59             | 0.11 [13] |
| 2013  | Selection generation-2                        | 11.80                  | 32.39             | 0.09 [14] |
| 2014  | Selection generation-3                        | 20.24                  | 20.24             | 0.12 [15] |
| 2014  | Field /farmer production trials               | : Conducted with farmers at seven districts | | 2 |
| 2015  | - Launching (Mutiara strain of African catfish) | : Officially launched through Decree of Ministry of Marine Affairs and Fisheries | | |
|       | - Dissemination                               | : As per 2017 more than 100.000 broodstock of Mutiara strain have been disseminated to 29 out of 34 Provinces in Indonesia | | |

4. Field Testing of Production in Farmer Level

The genetic gain obtained in a breeding program, represented by select individuals, mostly occurred in breeding nucleus facility, condition of which may differ from the real aquaculture condition in farmers’ facilities. In general, the differences may come in the form of differences in elevation of location and farming systems. Concerning African catfish farming in Indonesia, the elevation of location in which African catfish farming practices take place is highly variable. They ranged from nearly zero above sea level (ASL) to several hundred meters ASL in mountainous areas. Similar diversity is applied with farming systems, which mainly dealt with feed input, stocking density, and aquaculture facilities. While most grow-out farmers relied on fully commercial feed, some farmers, due mainly to limited financial support, took benefit either partially or totally from domestic or kitchen waste. Some other farmers also applied bioflock technology to improve feed efficiency. Concerning stocking density, although most farmers applied 100 fish/m², a lower (up to as low as 50 fish/m²) and a higher (500 fish/m² such as in bioflock system) stocking densities were also frequently applied, the option of which were related to feeding management. Eventually, diversity in the African catfish farming system was also associated with grow-out facilities. While earthen ponds...
have been the most widely applied, several other forms of confinement facilities were also found. They were a whole concrete pond, concrete pond with soil in the bottom, and plastic-lined structures. Due to these differences, there would be possibilities that the great performances of fish expressed in the breeding nucleus might not be expressed in different aquaculture environment. In order to obtain information on that aspect, field trials of grow-out involving farmers' collaborators have been conducted.

Mutia strain of African catfish seed, produced by breeding nucleus at RIFB was sent off to the farmer collaborators, to be raised to harvest size. The collaborators were asked to raise the strain along with their local seed as a comparison. They were emphasized to treat both groups in similar ways in any aspect of farming management, such as stocking density and feeding and water management. The farming systems implemented during field trials were set independently by respective collaborators. Seven selected collaborators, all were located around the breeding center, did the field trials. The localities of the collaborators (based on district) included Tegal, Cirebon, Indramayu, West Bandung, Karawang, Bogor, and Depok.

Results of field trials showed that Mutia Strain of African catfish performed better in growth, productivity, feed conversion ratio, and proportion of harvest size than its local strains counterpart. The magnitude of superiority varied with farming systems and locations. It was 10-40% better in growth, 15-70% better in productivity, 2-9 times higher in benefit-cost ratio, shorter growing period (45-60 days), lower feed conversion ratio (0.6-0.8 in nursery and 0.6-1.0 in grow out) and higher survival (60-70%) following challenge test against Aeromonas hydrophyla infection. This superiority was observed consistently in all locations at any farming system [19]. Although the field tests were not designed to explore the genotype by environment interaction, the fact that superiority of the Mutia strain occurred in every location and every farming system, it seemed that the phenomena of genotype by environment were not significant.

5. Putative Correlated Response to Selection for Growth Performance

Many breeding programs reported the presence of a correlated response, in which selection on one trait may affect other traits. This occurs when there is a genetic correlation among traits [20]. The term putative response to selection in this paper is used as the data to support it was not conclusive. No measurements were taken on the traits below, as the initial state of the trait before selection. They were not measured in the base population. Instead, they were measured at the end of the third generation of selection in the selected population and the population representing a part of the base population. Two traits, namely resistance to disease and tolerance to stress, are discussed in this review.

5.1 Resistance to disease

Farming of African catfish, as those with the farming of other fish farmed species, has not freed from disease problem. Primary disease attacking African catfish farming has been a bacterial disease caused by Aeromonas hydrophyla, which frequently caused disease problems during the juvenile stage. Evaluation of resistance to disease of the Mutia strain was carried out through a challenge test with Aeromonas hydrophyla at the dose concentration of 3,08 x10^8 CFU/ml. A hydrophyla is widely known as a significant disease infected African catfish, particularly during nursery stage. The evaluation showed that Mutia strain was much more resistant to disease than the local strain counterpart. For instance, following 24 hours of challenge test, the mortality rate of Mutia was 13.33%, while that of the counterpart strain was 58.33%. For a test duration of 60 hours, the counterpart Sangkuriang strain, one of the strains composing the base population, experienced total mortality. Conversely, only 30% of the selected Mutia strain died [21]. This result showed that selection for improved growth performance, using a composite base population as raw material, has not only improved the growth but also has improved the performance of other traits, namely resistance to disease.
5.2 Tolerance to Stress

Aquaculture, as an artificial environment, is profoundly different from nature, where fishes live in their natural condition. Living in high stocking density in cramped conditions, along with other artificial inputs (for instance, feed, handling) may induce stress to fish [22].

Farming-related handing activities such as stocking, grading may also induce stress. Fish allocated some portion of their energy to cope with stress leading to reduced energy available for other activity, including growth. As a result, the growth performance of less stress-tolerant strains may be lower than those of more stress-tolerant strains. The level of cortisol hormone has been widely used as a biological marker to measure stress-tolerant response in a biological system including fish [see, e.g., 23, 24-26].

Characterization of tolerance to stress in Mutiara strain resulted from the selective breeding program found that it was more tolerant than the counterpart Sangkuriang strain. This was indicated by a relatively lower level of cortisol in Mutiara strain (4.32±0.43 ng/mL) than that found in the counterpart strain (4.81±0.31 ng/mL). It is of interest to explore the factors that have contributed to the higher level of resistance to stress in this strain. One possibility of them could be the genetic composition of the base population, in which it has a broad genetic basis. It is of interest to know whether the relative stress-resistant trait was equally contributed by founder strains or a particular strain has contributed more than the others. A study in coral trouts *Plectropomus maculatus* and *Plectropomus leopardus* found that this trait seemed to be species-specific [27]. Whether the pattern of specific strain could explain the phenomenon is interesting to see.

6. Present Status of Dissemination and the Future African Catfish Breeding Program

To date, just three years following its launching in 2015, the Mutiara strain of African catfish resulting from the Research Institute for Fish Breeding, has been widely spread to nearly all over the country. A total of over 100,000 broodstock of Mutiara strain have been disseminated and are in use by farmers. At the end of 2017, It has been disseminated to 29 out of 34 provinces in Indonesia. It has been one of the most successful fish breeding programs. To serve the high demand, collaborations to multiply the broodstock of Mutiara strain have been made with Provincial Fisheries Service in Java Island, including West Java, Central Java, East Java, and Yogyakarta, provinces respectively. In addition to dissemination, efforts have also been made to improve the breeding program better.

A collaboration has also been made with Wageningen University and Research expert in fish breeding to allow the present breeding program to keep contributing significant genetic gain for the long term. As a result, an improved and more sophisticated breeding scheme by applying genetic evaluation of the best linear unbiased prediction (BLUP) method is underway. This subsequent breeding program is aiming at improving not only growth traits but also other economically important traits such as survival and hardiness. The results of this will be coming in further reports.

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