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TECHNIQUES OF CONTROLLING MIXED SEX TILAPIA AND THE PRACTICABILITY OF HORMONAL SEX REVERSAL IN CAMEROON

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ABSTRACT

Tilapia production has grown in recent years leading to the expansion of fisheries industry. Tilapia (*Oreochromis niloticus*) has the ability to grow fast, to breed in ponds, to adapt to shallow and turbid waters, to resist to diseases and to be flexible for culture and performance in different farming systems. These advantages have encouraged many culturists (small and large scale) to grow tilapia worldwide. However, one of the major drawbacks of fast growth in tilapia is early maturation. This often leads to stunted growth, low yield and unmarketable fish due to different sizes in fish (adults and offspring) competing with the initial stock for food. It has been argued that mono sex male tilapia culture may resolve most of these problems. All males are preferred because they grow faster than the females, they are more uniform in size, and are larger than the females who tend to use more energy for maturing gonads instead of growing. This project reviews mono sex male production techniques including, polyculture, manual sorting, hybridization, hormonal sex reversal, genetic male tilapia and triploidization by heat shock /pressure. Based on this assessment, oral administration of 17 a-methyl testosterone (MT) appeared to be the simplest and most reliable method to produce all male tilapia in hatcheries in Cameroon. This choice was based on the availability of equipment, costs and application of technique. However, as it appears from other studies, treatment of tilapia with MT should be restricted to the first month after first feeding i.e. 25-30 days. Precautionary measures should be taken to avoid direct release of hatchery water into the environment.

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1 INTRODUCTION

Aquaculture is a fast growing industry sector that has potential to reduce poverty by providing protein to human populations (FAO, 2014). Fisheries and aquaculture remain one of the vital sources of food nutrition, income generation and livelihood for people around the world today (FAO, 2016). Fish production has grown rapidly over the past fifty years; 59.4 million tons in 2014 as compared to one million tons in the early 1950s (FAO, 2014). Today aquaculture provides roughly half of all fish for human consumption, and also has the potential to reduce fishing pressure on certain fish stocks.

After salmon and carp, tilapia is the third most cultured fish in the world (Tveteras, 2016). Tilapia culture started about 4000 years ago as depicted in the Egyptian history (FAO, 2005-2007). This practice was distributed to other countries around the world later on. The introduction of Nile tilapia (*Oreochromis niloticus*) in China in 1778 was a turning point for global tilapia production, and China consistently produces more than half of the global production of tilapia annually. In 2000, global output of tilapia was 1 million tons, and in 2005 it was more than 2 million tons (FAO, 2014). The main producing countries of tilapia in the world include: China, United states of America (Florida and Texas), Latin America (Argentina, Brazil, Chile and Peru), European union (Netherlands and Belgium), Asia and Egypt.

Aquaculture started in Cameroon in 1948 with the culture of tilapia *(Oreochromis niloticus*) and this was followed by Catfish (*Clarias gaerepinus*) in 1990 and later on common carp (*Cyprinus carpio*) (FAO, 2005-2006). Although other species of fish are being reared in Cameroon, Nile tilapia (*Oreochromis niloticus*), native of African continent, is well adapted to local conditions such as extreme temperatures and low levels of dissolved oxygen. The spawning process of tilapia starts when the male creates a territory, digs a spawning nest and guards its terrain and invites the female. Immediately after spawning and fertilization, the female collects the eggs into its mouth and leaves the nest for incubation and brooding. During brooding the females eat little or no food, between1 to 2 weeks depending on the temperature. The females are obliged to guard the fry in case of any danger until the yolk sac is absorbed. The number of eggs depends on the size of the female. In each spawning batch, female of 100g will produce 100 eggs while those from 600-1000 g can produce 1000 to 1500 eggs (FAO, 2005-2007).

The system of culture in Cameroon includes earthen ponds, concrete tanks, and cages. Plastic tanks and recirculating systems are used in research station. The annual production of fish by small scale farms is between 2-5 tons of fish per hectare (extensive technique) and about 16 tons per hectare for large scale farms (semi-intensive) (Tekwombou, 2014). The most common practice is polyculture of Nile tilapia with another species such as African catfish which is usually done in earthen ponds (Pouomogne & Pemsi, 2008). The aim of polyculture is to take advantage of many natural foods available in ponds and to control tilapia recruitment. Fish are usually sold at the pond site to family members and community at large, unsold fish are usually taken to restaurant or smoked.

Capita consumption of fish in Cameroon is 17.9 kilogram per year (Tekwombou, 2014). In spite of this increase, fish is still preferred by most Cameroonians to other source of protein. When compared with meat, chicken, beef and pork, fish is cheap and can be affordable by the poor and the underprivileged (Pouomogne & Pemsi, 2008). In recent years, Cameroon government has imported a lot of fish from other countries. This is because the supply of fish does not meet the high demand due to growing population. It is estimated that more than 120,000 tons of fish is imported annually for the past years in Cameroon (Pouomogne & Pemsi, 2008). Sustainable production of tilapia in Cameroon could decrease the total importation of fish needed and provide fish protein to populations in villages.

To change this situation, the government has put in place a strategic framework for the development of sustainable aquaculture with the assistance from FAO and World Fish Centre (Moehl *et al*., 2005). Also, government projects and programs such as the National Agricultural Extension and Research Projects (PNVRA), the Amelioration of Competitiveness of Family Agro-Pastoral Farming Programm*e* (ACEFA*)* and Livestock and Fisheries Development Project (LIFIDEP) in North West have been providing technical advice, financial and material through their extension services to the farmers. Through these projects and programs, new ponds are constructed, old and abandoned ones being rehabilitated, and farmers are trying to group themselves into cooperatives to revitalize the sector (Pouomogne & Pemsi, 2008). It is therefore possible that aquaculture will grow in the near future specifically, if high quality fingerling is readily available in Cameroon (Tekwombou, 2014). Cameroon has good climatic condition suitable for culturing many warm water species, good soil texture for pond construction and natural inland water covering more than 40.000 square kilometers and fresh water for Aquaculture (Brummett, 2007). There are about 5260 fish ponds spread over 5.357.000 m^2 (area of Cameroon) and to satisfy the need of these farmers, about 27 hatcheries are required in the eight regions of Cameroon that will be able to produce 44 millions fingerlings to farmers (Tekwombou, 2014).

1.1 Justification of the study

However, one challenge of tilapia farming in the world and Cameroon in particular is early maturation (Ajiboye *et al.*, 2015). Controlling reproduction of tilapia in ponds has been a major challenge in tilapia aquaculture (Omasaki *et al.*, 2016; Ferdous & Ali, 2011; Fuentes-Silva *et al*., 2013). Early maturation often leads to stunted growth, low yield and unmarketable fish due to different sizes in fish (adults and offspring) (Popma & Masser, 1999). Due to excessive recruitment, most of the tilapia produced in mixed sex culture are skinny and bony because offsprings compete with adults for food, there is less dissolved oxygen available, and they are affected by greater releases of ammonia and faeces. As a result, it is hard to produce large and uniform sizes of tilapia which is desired by the consumers. All male tilapia or sterile fish are preferred because they grow faster. Males are more uniform in size and are larger than the females who tend to use more energy for maturing gonads instead of growing. Rendering the fish sterile can help to increase growth rate and to reduce energy put into reproduction (i.e. growing gametes). Various techniques have been used to control mixed sex tilapia populations in other countries in the world and Africa for aquaculture purposes (Dauda *et al*., 2014; Ferdous & Ali, 2011; Chakraborty & Banerjee, 2010). These techniques include: the practice of polyculture, gender sorting, hybridization, hormonal treatment, and genetic male tilapia (GMT). These techniques have their advantages and disadvantages that may differ according to countries, depending on the scale and the type of farming systems. Unfortunately, in Cameroon, there is still a problem of awareness and education since farmers and technicians lack knowledge about the reproduction of all male tilapia and how to control it, even though this has been researched in many countries. The goal of this project was to review all the techniques used in controlling mixed sex tilapia in other countries and identify the best method that can be practiced in

Cameroon. This method will be passed on to the directors of the various hatcheries through training to enhance their knowledge for sustainable aquaculture of tilapia in Cameroon.

1.2 Specific objectives

- To review worldwide techniques for controlling mixed sex tilapia
- To identify the best method for controlling mixed sex tilapia in Cameroon
- To access the impacts of this method on the fish, the consumers, the culturist and the environment.
- To produce a training guideline for the best method for Cameroon

2 METHODOLOGY

To achieve the first objective, a systematic review of techniques in controlling mixed sex tilapia was carried out using peer-reviewed scientific publications. Scientific papers were selected from science direct which is a database for scientific and medical research using specific key words. These keywords were: “tilapia” and “mono sex” and resulted in 419 papers. This number was reduced to 45 (44 articles and one book) if these two words were present in the abstract, title and/or keywords of each peer reviewed sources. From the 44 papers, 15 relevant papers were identified including articles and reviews. Because science direct does not cover every journal of interest such as fish biology, more papers were chosen from google by searching specific key words not found in the previous research, and references found in these 15 papers from science direct search were used. In this search, 25 relevant papers were added making a total of 40 articles and reviews used to address my first objective. From the 40 papers, information like the description of the techniques, the advantages and disadvantages of each of them and the results were sorted. This information was used to substantiate the different techniques, to identify the best method for controlling mixed sex tilapia in Cameroon, and also to come out with the training guidelines.

Apart from the scientific papers, information was collected from the three Directors of aquaculture stations (Joseph Arsène Obama, Coastline Regional coordinator, Littoral; Joseph Etienne Mballa Mamga, Regional coordinator for the promotion of aquaculture entrepreneurial (PPEA) activity in the Center Region, and Alain Eloundou Regional coordinator for South) from Cameroon. Jean-Francois Baroiller, a fish biologist at “Centre de Coopération Internationale en Recherche Agronomique pour le Développement” (CIRAD) in France was also contacted; he is currently writing a book on the control of mixed sex tilapia in the world.

3 REVIEW OF WORLDWIDE TECHNIQUES FOR CONTROLLING MIXED SEX TILAPIA

Controlling reproduction has been one of the biggest challenges in tilapia culture over the years. This has necessitated the development of various methods to overcome this challenge. Different methods have been used such as: polyculture, manual sorting, hybridization, genetically modified tilapia (GMT), hormonal sex reversal and triploidization. These methods are briefly described below.

3.1 Polyculture

Tilapias are frequently cultured with other species to take advantage of many natural foods available in ponds and to control tilapia recruitment (Little & Edwards, 2004). This system is called polyculture. In polyculture, a combination of species with different feeding niches are used to increase production without a corresponding increase in the quantity of supplementary feed (Rakocy & McGinty, 2005). This means that any food not eaten by the tilapia will be eaten by another species and will not be wasted. Polyculture of tilapia with predatory fish such as snakehead (*Ghana striata or Clarias gariepinus*) does not prevent overcrowding but can reduce recruitment by eating the younger tilapia (fry and fingerlings) produced by spontaneous and uncontrolled reproduction of larger tilapia and allows the fish to have more homogenous size at the end of the production cycle (Little & Edwards 2004; Phelps & Popma, 2000). Also, adding catfish to tilapia in the same pond would not add to the cost of production because the predator eats the fry and the fingerling and equally feed on the same feed with tilapia. When sold, the yield and net profit will increase.

Stocking density of the predator species may be hard to determine. Predators must not be stocked at the same density as tilapia to prevent them from eating the original stock (Little & Edwards, 2004). The number of predators required to control tilapia recruitment in cultured ponds depends primarily on the maximum attainable size of the predator species, the ability of the predator to reproduce, and the number of mature female tilapia. Also, predators should be introduced once tilapia start breeding. Overall, the disadvantage of polyculture is that as predators grow, they tend to eat larger tilapia, especially in a pond where feeding is not provided as the predator have the ability to move from one pond to the other (Little & Edwards, 2004). In Cameroon, tilapia is usually farmed in polyculture with African cat fish (*Clarias gariepinus*). Polyculture increases productivity by a more efficient utilization of the ecological resources in the pond. Tilapia, *O. niloticus*, is an omnivorous fish and African catfish is a predator targeting young tilapia (fry and fingerlings).

3.2 Manual sorting

Based on the anatomy of the genitals, it is possible to sort tilapia into males and females when they have reached 50-80 g (Mair & Little,1991). The females and males can easily be sorted through the anal papilla by visual inspection (Omasak *et al*., 2016) or with the aid of dye (Fortes, 2005; Fuentes-Silva *et al*., 2013). The male genital papilla is simple and smaller with two openings: the urogenital opening where the milt and urine are excreted and the anus (Figure 1). The male genital papilla has a cone like shape located behind the anus, whereas the female has a large and wider papilla with three openings: the anus, the urethra and the oviduct where the eggs pass. However, the sorting of sexes can be done when tilapia have reached adult stage or when their secondary sexual characteristics are well developed. The reliability of sex sorting depends also on the skill of the workers and the size of the fish to be sorted. Although manual sex sorting is feasible, it is tedious and can stress the fish through too much handling (Beardmore *et al*., 2001; Popma and Lovshin, 1996), and it is difficult even for skilled workers to achieve 90% of success in sex sorting, as a result, reproduction is rarely controlled. This method may have negative impacts on economic returns because you need to employ skilled workers or train them at a cost, and female fish are all discarded after sorting stage (Beardmore *et al*., 2001; Popma & Lovshin, 1996). The major disadvantage of this method is that it is labor intensive, and subject to human error (Beardmore *et al*., 2001). Consequently, this method is generally applied at subsistence level and in hatchery during brood stock selection, where fish populations are normally small (Fuentes-silva *et al.,* 2013). Figure 1 illustrates the male and female reproductive organs. On the left is the female's reproductive organ with three openings the anus, the oviduct and the urethra while on the right is the male organ, with two openings, the urethra and the anus.

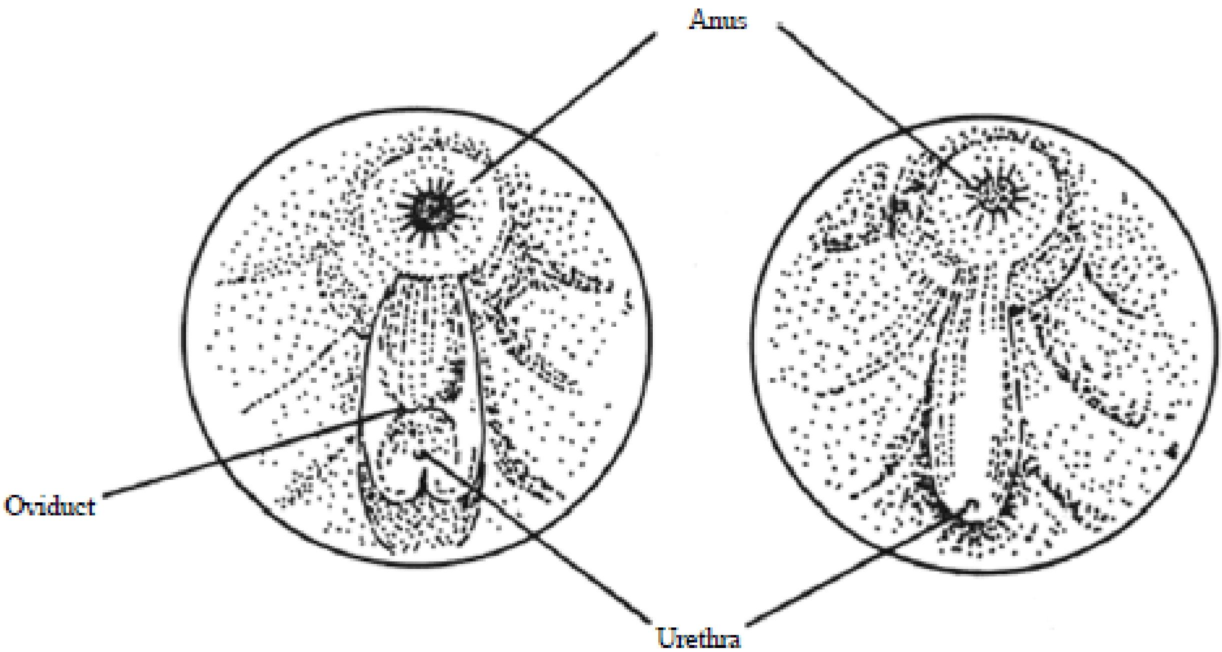

Figure 1: Male and female genital organ of tilapia (FAO, 1976)

3.3 Hybridization

Hybridization is the mating of two different species to produce a hybrid (Beardmore *et al*., 2001). The aim of hybridization is to produce sterile offspring thereby reducing unwanted natural reproduction in grow out ponds. Hybridization is not only used to manipulate sex ratios or produce sterile fish, but also to increase growth rate, improve flesh quality and increase disease resistance (Bartley *et al*., 2001). Because different species of tilapia belonging to niloticus genus are mouth brooders, it became possible to carry out manipulation of the eggs and milt to produce hybrids. Thus, reducing the problem of either fish not wanting to cross with each other or because they do not look alike. With this technique, it is difficult to achieve 100% sterility of a population because of difficulty in maintaining pure parental stocks that consistently produce 100% sterile offspring, and difficulty in producing adequate number of fry due to spawning incompatibility between parent species (Popma & Lovshin, 1996). Hybrids may still require manual sorting of sexes since all crosses do not produce 100% sterile offspring. Also, two species of tilapia must be maintained, and raised separately instead of one. Table 1 below summarises all the hybrid combinations of different tilapia used to producing sterile offspring.

Table 1: Hybridization of different tilapia species known to produce sterile tilapia population (Beardmore *et al.*, 2001).

Males	Females	Comments
Oreochromis niloticus	*Oreochromis aureus*	Applied commercially but results inconsistent
Oreochromis niloticus	*Oreochromis macrochir*	
Oreochromis niloticus	*Oreochromis urolepis hornorum*	Majority of broods are all-male Some commercial application
Oreochromis niloticus	*Oreochromis variablis*	All progenies monosex
Oreochromis mossambicus	*Oreochromis aureus*	
Oreochromis mosambicus	*Oreochromis urolepis hornorum*	All progenies monosex
Oreochromis spilurus niger	*Oreochromis urolepis hornorum*	All progenies monosex
Oreochromis spilurus niger	*Oreochromis macrochir*	
Oreochromis aureus	*Oreochromis urolepis hornorum*	
Oreochromis zillii	*Oreochromis andersonii*	All progenies monosex

3.4 Hormonal sex reversal

Challenges associated with manual sorting of sexes and hybridization techniques have led to another technique known as hormonal sex reversal. Hormonal sex reversal is the administration of hormone through dietary supplementation (Carrasco *et al*., 1999). The process starts with the androgen dissolved in alcohol and incorporated in feed and administered to fry (Beardmore *et al*., 2001; Singh, 2013). This technique is usually applied when the fry are sexually undifferentiated after hatching or up to the swim-up stage as suggested by several authors (El- Greisy & El- Gamal, 2012; Rowell *et al*., 2002; Jensen and Shelton, 1979). Hormonal sex reversal is more effective, less cumbersome and leads to higher growth rate than other methods. Studies have shown that 60 mg/kg of food dose from the 7-28 days allows to obtain 95-97% of males as compared to a 35 mg/kg dose for 60 days that leads to 100% (El- Greisy & El- Gamal, 2012; Singh, 2013). The doses per kg of feed use when administered orally varies from one author to the other from 15 mg to 80 mg. Several authors suggested that this method leads to higher growth, uniformity in size at harvest and sexual behavior reduction (Beardmore *et al*., 2001; Sayed *et al.*, 2016; Little *et al*., 2003; Omasaki *et al*., 2016).

On the other hand, this technique has been criticized by many. Hormone doses are not uniform in feed making estimate of amount very difficult. Excessive hormone doses can lead to sterility in fish (Beardmore *et al*., 2001). Also no guidelines relating to frequency, timing of feeding has not been developed and this can lead to failure. This technique has been criticized based on the fact that many believe the residual effect of such hormone has harmful side effect (Singh, 2013). In this study 35 mg of (MT) per kg of feeds was used for 60 days that enables to obtain 100% males which was a very long period. On the other hand, other studies have shown that the used hormones have no side effect if the right doses and quantities are respected (50 to 60 mg/kg) within the period of 28 to 30 days. Also, that hormone is rapidly metabolized and excreted after ingestion. They also suggested that hormone levels in tilapia falls to normal level after hormonal feeding was stopped (Macintosh, 1995; Rowell *et al*., 2002; Guerrero, 2008).

This hormonal sex reversal method can also be done through immersion of fertilized eggs or fry sac. Immersion techniques have also been applied successfully (Cagauan *et al.*, 2004). Success of immersion depends on the hormone concentration and duration of immersion. In hormonal concentration of 800 unit per gram per litter (u g-l) 99.33 % of males were obtained after 96 hours. The duration of the treatment and cost of the hormone is low when compared to oral administration.

Sex reversal by immersion is a relevant method because the risk of workers coming in contact with the hormone is minimised. On the other hand, this technique cannot be applied to all species especially those that filter their feed like the Nile tilapia when dealing with fry and Chinese carp (Beardmore *et al*., 2001).

However, the use of hormone for sex reversal has been criticized by many (Singh, 2013) due to the possible impact on health and environment. It is assumed that the incorporation of MT in feed for sex reversal leads to the waste water leaking into the environment from hatchery where it is being used.

3.5 Genetically modified tilapia (GMT)

As a result of the problems involved in the application of hormones to produce all-male tilapia, an alternative strategy has been put in place known as the genetic male tilapia (GMT) (Abucay *et al*., 1999). The aim of this technique is to produce super male brood stock for the production of genetically modified male tilapia. This involves a genetic breeding programme combining hormone for feminization (Jensen and Shelton, 1979; Rowell *et al*., 2002; Ezaz *et al*., 2004). This technique involved lots of progeny feminization testing, followed by mating with normal male to generate novel YY genetically male tilapia (Ezaz *et al*., 2004; Beardmore et al., 2001). Males from this batch when mated with female tilapia produce all male tilapia offspring. Compared to the hormonal sex reversal method super male technology is believed to be more feasible on a commercial scale, and is environmentally friendly since use of hormones is limited to brood stock and no hormonal residues are detected in consumed fish. On the other hand, the technique can be complex, time consuming, tedious, labour intensive and still requires sex hormones at its initial stage (Mair *et al*., 1991). It is only suitable for homogametic species (XX/XY) (Beardmore *et al*., 2001). Thus, dissemination of information about its current application is limited especially in poor communities worldwide. Figure 2 shows the different stages of producing the (YY) super male and all male tilapia (XY).

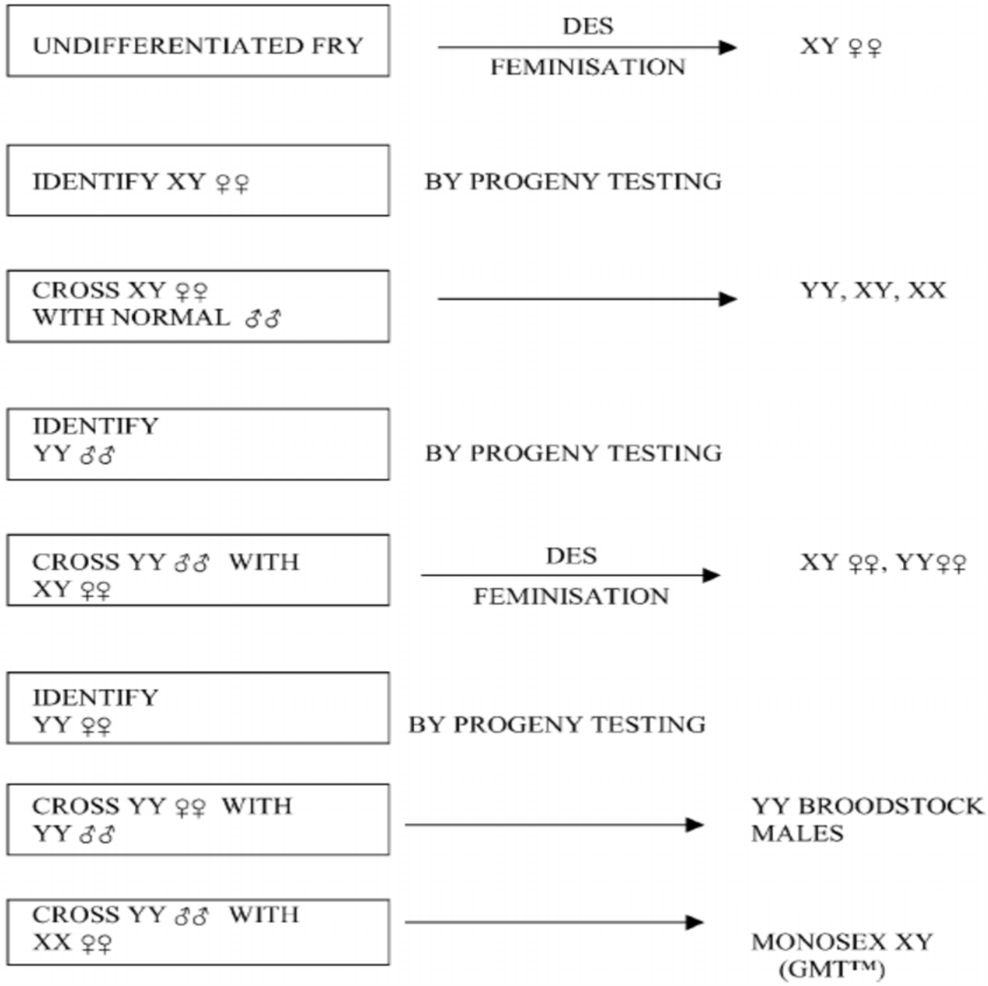

Figure 2: Schematic diagram for mass production of super male (YY) and all male (XY). (Beardmore *et al*., 2002)

3.6 Triploidization by Heat shock \ Pressure

Triploidization is a method to obtain sterile fish (Abucay *et al*., 1999). This is induced by the application of heat shock or pressure at specific time and duration after egg fertilization to disrupt normal extrusion of the polar body from eggs during meiosis as illustrated in several studies (Abucay *et al*., 1999; Baroiller and D‘Cotta, 2001; Desprez and Melard, 1989). It occurs when the eggs are exposed to pressure shortly after fertilization to hinder extrusion of the second polar body or cell division pushing the fish to have three set of chromosomes instead of two (Baroiller and D‘Cotta, 2001). Due to the genetic condition of triploid fish, germ cells cannot undergo meiotic divisions correctly. Consequently, triploid fish are usually genetically sterile, showing either gonadal sterility (lack of gonadal development of testis and ovaries) or gametic sterility (lack of gamete production) in both males and females (Abucay *et al*., 1999). In addition, because triploid fish are sterile, it has been considered that growth performance will improve but unfortunately it results to more females than males (Abucay *et al*., 1999). The advantage of this method is that it takes a very short time of about 30 to 40 min after fertilization depending on the species. Although this method is environmentally friendly, some embryos are still diploid (Abucay *et al*., 1999), there is poor survival rate due to the fragile nature of the eggs. This method cannot be feasible to all species especially those that filter their feed when dealing with fry.

Table 2 summaries the advantages and disadvantages of different techniques of producing all male tilapia. Based on this table, the best method for controlling mixed sex tilapia for Cameroon will be identified.

Table 2: Advantages and disadvantages of techniques for controlling mixed sex tilapia in worldwide aquaculture.

Method	Advantage	Disadvantage	Reference
Polyculture/ Police fish	-reduce recruitment -food not wasted -more yield and profit, two species are farmed and sold, adult tilapia grow better	-balancing stocking density -predation of the police species on all size tilapia	(Little and Edwards 2004) (Phelps and Popma 2000) (Rakocy and McGinty 2005)
Manual sorting	-environmentally friendly	-labour intensive -costly: both sexes need to reared until visual sexing -sorting errors -waste of feed - low % of success	(Beardmore *et al*.,2001) (Popma & Lavshin, 1996) (Omasaki *et al*., 2016) (Fuentes-silva *et al.*,2013) (Fortes, 2005) Popma and Lovshin (1996)
Hybridization	-sterile fish are produced	-two species must be maintained -reproduction incompatibility -difficulty to maintain pure parent stock -error in identification of brood stock	Popma and Lavshin (1996) (Beardmore *et al*.,2001) (Bartley *et al*., 2001)
Hormonal sex reversal by dietary implementation	-simple to apply - 98-100% male -female not discarded	- could have environmental impacts -consumer's perception -health impacts on the culturist -lack of uniformity of the doses of hormone	El- Greisy and El-Gamal (2012) (Beardmore *et al*.,2001) (Carrasco *et al*.,1999) (Singh 2013) (Omasaki *et al*., 2016) (Rowell *et al*.,2002) (Jensen and Shelton 1979) (Sayed *et al*.,2016) (Guerrero 2008)
Hormonal sex reversal by immersion	- reduce risk of workers coming in contact with hormone	-cannot be applied to all species	(Beardmore *et al*.,2001) (Cagauan *et al*., 1995)
Genetic modify tilapia(GMT)	-environmentally friendly -no hormonal residues are detected - strain is also not affected normal genotype	-complex -time consuming -Tedious -labour intensive -required hormone at initial stage	(Abucay *et al*., 1999) (Baroiller *et al*., 1996) (Rowell *et al*.,2002) (Ezaz *et al*.,2004) Desprez and Melard (1989) (Mair *et al*.,1991)
Triploidization by heat shock/pressure	-environmentally friendly -short time 30-30min	-not feasible in all species -some embryos still diploid -need equipment -lower survival rate	(Abucay *et al*., 1999) (Baroiller *et al*., 1996) (Baroiller and D'Cotta, 2001) (Desprez and Melard 1989).

4 IDENTIFICATION OF THE BEST METHOD FOR CONTROLLING MIXED SEX IN CAMEROON.

The identification of the best method for Cameroon was based on three criteria: availability of equipment, costs, and application of technique. Manual sorting of fingerlings is extremely laborious and expensive since the fish need to be fed, workers need to be trained on the skill of sorting and end up discarding the females. The method is stressful and reliability is low due to human error. Given the investment involved in the training and high risk of inaccuracy, the technique cannot be feasible in Cameroon.

Hybridization is not common in all species and it is difficult to maintain pure parental stock used to produce sterile offspring. It is difficult to produce larger number of fry due to spawning incompatibility. These are some of the reasons why this method cannot be applied in Cameroon. For the YY super male and GMT, a breeding program is needed and lots of progeny testing is involved making the methods complex, laborious and time consuming. There is no breeding program in Cameroon and for these reasons it cannot be applied in Cameroon.

On the contrary, hormonal sex reversal by oral administration of a synthetic hormone is easy to apply, and hormone can be ordered from other countries. About 98-100% of all males are achieved. Treatment is for the first one month and the dosage 25-60 mg per kg of feed. This means that traces of the hormone cannot be seen in the flesh after five months of rearing. Similarly, studies of sex reversal are done in earthen ponds which is the farming system practiced in Cameroon. Overall cost is not high. Hormone cost is 45300 CFA (Central African Franc) per gram in Asia. There is no need for laboratory analysis and equipment. Based on these assessments, the technique of sex reversal by oral administration of MT can be the best technique for Cameroon. The technique will be applied in the aquaculture stations where all the risks involved in hormone use will be controlled.

5 ASSESSMENT OF THE IMPACTS OF HORMONAL TREATMENT BY DIETARY IMPLEMENTATION

Although many studies have shown that hormonal sex reversal is one of the best techniques of controlling mixed sex tilapia population, the use of hormone in fish has been criticized by many who believed that it may pose health risk to workers, consumers, and the environment (Singh, 2013, El-Sayed *et al*., 2012). On the other hand, several authors have shown that MT is not harmful to humans (Megbowon & Mojekwu, 2014; Macintosh 1995, Rowell *et al*., 2002; Guerrero 2008).

5.1 Description and Uses of 17α -Methyl Testosterone

The use of MT in tilapia farming is not new. Macintosh (1995) found that MT started to be used in the late 1960s, when researchers tested several hormones in order to produce single sex tilapia to overcome the problem of mixed sex tilapia production in grow-out ponds. MT is a synthetic androgen steroid usually dissolved in alcohol and incorporated in feed during gonads development (Beardmore *et al*., 2001; Singh, 2013) resulting in the production of 100% phenotypic males. It promotes both muscle growth and the development of male and female sexual characters (El-Greisy and El- Gamal, 2012). Endogenous testosterone is produced by the testicles in males and in much lower quantities by the ovaries in females (Macintosh, 1995). Macintosh (1995), Beardmore *et al*., (2001) suggested that excessive doses of some hormone can lead to sterility of the female fish. In countries where the use of hormone is allowed, androgens and estrogens steroids are commonly used in agriculture to promote weight gain in cattle and sheep. The dosages of hormone applied in human medicine, or livestock production is greater as compared to the quantity of 0.02 mg used in fish which is applied to early fry for about 30 days and are reared for at least five months. According to the chief of aquaculture station in Douala Cameroon, 1 g of synthetic hormone from Asia costs 45300 CFA francs.

5.2 Potential risks associated with the use of methyl testosterone in tilapia farming

The risks of MT in tilapia farming can be divided into three main aspects: a) risks on the fish for consumers, b) risks to fish farm workers, c) risks to the environment.

5.2.1 Risk on the fish for the consumer

Research studies have shown that MT does not accumulate in the flesh of tilapias (Rowell *et al*., 2002; Megbowon and Mojekwu, 2014; Macintosh, 1995). Other authors found that hormone is rapidly metabolized in liver, kidney and cleared from blood plasma excreted within a short period after ingestion (Rowell *et al*., 2002). Also, hormone levels in tilapia falls to normal level 5 days after cessation of treatment (Guerrero, 2008). The feeding of fry with MT when the gonads are still undifferentiated for 28- 30 days did not show any trace of hormone in the flesh (El- Greisy & El-Gamal, 2012). Based on these scientific evidence, it is clear that MT cannot be found in the flesh of fish after five months of rearing. The recommended best practice is to restrict tilapia MT treatment to the early fry stages, specifically to the first month from the time the fry are free-swimming/first feeding and to limit the dosage of MT a maximum of 50-60 mg MT/kg fry feed as suggested by Guerrero (2008).

5.2.2 Risk of methyl testosterone to culturist

For MT purchases in powder or tablet form (Beardmore *et al*., 2001; Singh, 2013), the process starts with the androgen dissolved in alcohol and incorporated in feed which is offered to tilapia fry three to four times per day during the hormone treatment period (Singh, 2013). Farm workers or culturists can come into contact with MT in two ways: (a) when it is being added to tilapia fry feed, (b) when MT treated feed is being administered to tilapia fry in hatchery tanks or hapas. The risk to fish farm workers can be minimized by following the standard for handling such substances. Use of protective surgical gloves and face mask are strongly recommended to reduce the risk of inhaling the hormone or entry through the skin contact (Beardmore *et al.*, 2001; Singh, 2013).

5.2.3 Risk of releasing methyl testosterone in the environment

Today there is an increasing concern that the hormone wastewater from tilapia hatchery that is discharged into the environment can cost environmental hazard (Megbowon and Mojekwu, 2014; Macintosh, 1995). These authors also suggested that the major source of waste into the environment are animal manure and sewage out flow and agricultural waste water. Laboratory studies on sedimentation and suspended study have revealed that 1 week after cessation of MT feed administration, 57% of sediment and 78% suspended MT is lost (Mlalila *et al*., 2015). Other authors suggested that the global production and discharge of pharmaceutical active steroid to the environment from aquaculture is about 100kg of MT annually while about 33 tons of estrogen and 7.1 tons of androgens are discharged annually from animal in EU (Megbowon & Mojekwu 2014). In this perspective, compared with those from other sources, the waste from hatchery is small and represents only a very small fraction that is discharged into the environment. However, precaution should be taken when discharging waste into the environment. It is therefore recommended that all treatment of fish with MT should be carried out in the hatchery where the dose, the timing and the discharge of waste will be well controlled.

Another major concern is the tilapia escape in the environment. Tilapia fry that escape from hatchery where MT is administered will be male and consequently, their mating in the wild with closely related species may lead to hybrid that may threaten or eliminate pure wild populations that are vital tilapia genetic resources for future breeding programs for aquaculture. This risk can only occur in areas with native populations of closely-related species. However, barriers should be constructed on the inlets and the outlets of tilapia culture enclosures e.g. tanks and ponds. Such that fish cannot pass through the barriers, the barrier on the inlets and outlets should be equipped with net mesh or grills / screen to retain the stocked fish.

6 TRAINING GUIDELINES FOR PRODUCING ALL MALE TILAPIA THROUGH HORMONAL INTERVENTION FOR HATCHERY TECNICIANS

6.1 All male fingerlings production

Production of all male tilapia starts with selection of male and female brooders. This usually takes place in tanks, ponds and hapas. A hapa is a net that is designed like a mosquito net usually placed in a tank or pond which is used to control breeding process. After selecting the males and females using their genital organs, they are kept in separate tanks or hapas and fed with high protein diet. This is followed by an examination of the female to verify if the eggs are mature. If there are signs that the eggs have matured, both sex are brought together into one hapa in the ratio of 3:1 (three females and one male per meter square).

6.1.1 Characteristics of good brood stock

- Good body conformation traits (small head, body ratio, deep body, relatively thick, good dress percentages)
- Free from pathogens and diseases
- Bright even colour
- Good egg production (Around 500 eggs per spawning)
- From good genetic pool (genetic diversity retaining)
- Body weight 350-500g

6.1.2 Fry production: Eggs collection and cleaning

The male prepares the spawning nest where the females lays the eggs. The spawning cycle lasts approximately 19 to 21 days. After fertilization, the females collect the eggs in their mouth for incubation lasting from 3-5 days depending on the temperature.

At the end of this period, the pond is drained and the brood stock are removed by lifting the hapa mesh. The mouths of females are checked from the 8th to the 10th days by opening with fore finger and wash any eggs into receptacle by back and front movement of fish. Eggs are cleaned of debris with enough water and taken to the hatchery for incubation and hatching. From the 12th to the 14th days, the eggs hatch and tiny fry with yolk sac emerge and eventually the yolk sac is absorbed from the 10 to the 16 days after fertilization and the fry beginning to swim to the outer edges of the hapa. At this point the fry are scooped using the hand net to the nursing ponds where they are fed with the about (28-30 days). Figure 3 is the summary of reproductive cycle of tilapia in pond, tank and hapa.

6.2 Hormonal feed preparation (Guerrero, 2008)

6.2.1 Input requirement

- Absolute Alcohol (90 -95 %)
- Hormone: 17 alpha Methyl testosterone
- Powder Starter Feed (40 % protein)
- Vitamin Premix

6.2.2 Feed preparation (El- Greisy & El-Gamal, 2012, Megbowon & Mojekwu, 2014)

- The sex-reversal diet is prepared by an alcohol evaporation method.
- Weigh 50-60 mg of the hormone, alpha-methyl-testosterone in a plastic container.
- Dissolve in a one-litter solvent of methanol or ethanol and stir with a glass rod.
- Weigh one kilogram of Powder Starter Feed into a bowl.
- Add hormone little at a time and mix thoroughly.
- Expose mixture to air in a shade on a plastic sheeting or bag to evaporate alcohol and dry feed.
- Store in a plastic container in a fridge to delay bacterial or fungal contamination.

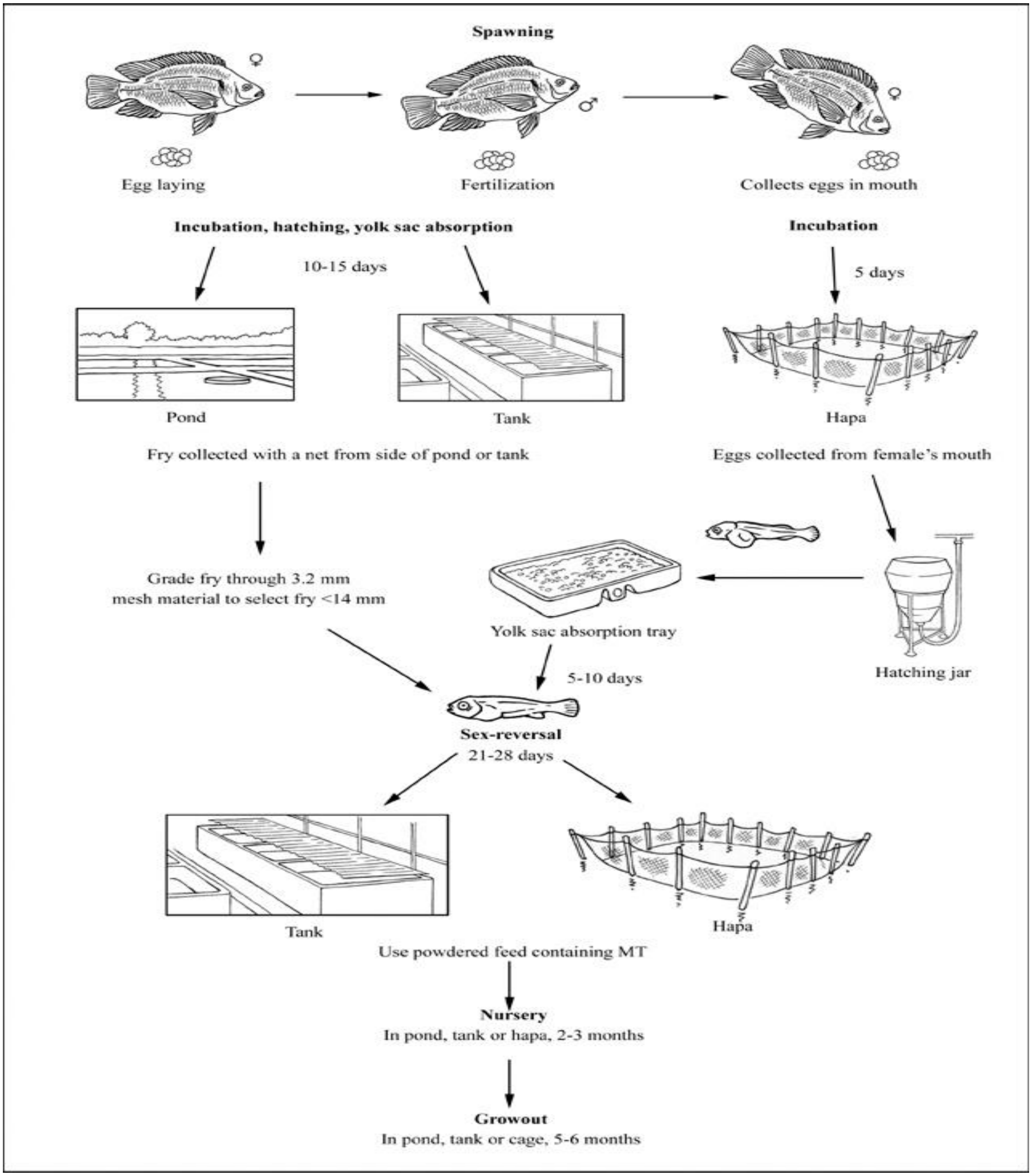

Figure 3: Summary of production cycle of Oreochromis niloticus in pond, hapa and tank. (FAO, 2005).

6.2.3 Fry treatment (FAO, 2005-2007)

- After hatching scoop fry using the hand net to a container into a treatment tank where they are chemically treated for diseases and parasites, which they may acquire during hormonal treatment period.
- Transfer fry to nursing pond, tank or fine meshed hapas.
- With the temperature of 23-26°C, feeding ration at the beginning should be at the rate of 20% body weight 4 times daily for the first two weeks. After that, gradual reduction down to 10% of the fish body weight per day till the end of treatment (28-30 days).
- Weekly sampling of the fry should be done to assess the growth and to determine feeding. Upon completion of the sex-reversal treatment, fry should be stocked in small nursery ponds for rapid growth. Prior to their stocking in production ponds they should be fed with floating or sinking feed. Make sure that formulated feed contains all nutrient elements that fish should need to grow well in captivity (40 % protein). Cover net to prevent predators and birds. If sex-reversal is conducted in hapas, the feed must be of a consistency that allows it to float. Otherwise a considerable amount of feed would be lost as it settles through the bottom of the hapa. Sex-reversed fry reaches an average of 0.2 g after 3 weeks and 0.4 g after 4 weeks. The average efficacy of sex-reversal ranges from 95 to 100 % depending on the intensity of management.

7 CONCLUSION

Oral administration of MT is the simplest and more reliable method to produce all male tilapia. All male tilapia grows larger and more uniform size than mixed sex or all-female tilapias. It is very effective on the Nile tilapia, *O. niloticus*. Although the legal status of the use of hormone in aquaculture vary from country to country, scientific evidence has proven that MT has no human health risks, provided the recommended dosage and duration is respected. Also, the amounts of MT consumed by tilapia fry throughout treatment are insignificant compared to the levels of testosterone produced by both men and women, and consumed in other foodstuff, especially meat and dairy products. Besides, digested hormone is rapidly metabolized and excreted after cessation of the treatment. Thus, MT is not noticeable in adult tilapias after five months of growth. Moreover, there are no reported health effects among workers at tilapia farms where MT is used.

Concerning the environmental impact, the amount of MT that may be entering into the environment are lower than the hormones being released from agricultural wastes and sewage. There are no reported health effects among workers at tilapia farms where MT is used, but standard procedures for dealing with all chemicals and pharmaceuticals should be applied to MT as a routine precaution. However, the precautionary measures should be taken to avoid direct release of hatchery water into the environment by utilizing gravels and sand filters, plus a shallow vegetated pond or enclosed wetlands, to receive and hold the hatchery wastewater for several days before discharging into the environment. Finally, in intensive fish production, the closed circuit systems or controlled aquarium conditions can be adopted for best management practices for responsible aquaculture.

8 RECOMMENDATIONS

- The project will be presented to the Ministry of Livestock Fisheries and Animal Industries (MINEPA).
- Verification should be made to make sure that Cameroon rules and regulation does not prohibit the use of hormone.
- A pilot phase for the production of mono sex should be carried out in one of the aquaculture station to evaluate the effectiveness of the MT.
- Analysis should be carried out in National Veterinary Laboratory (LANAVET) to see if hormonal residual is found in the flesh of the fish.
- Treatment of tilapia with MT should be restricted to the early fry stages, specifically to the first month from the time the fry absorbed their York sac for 25-30 days.
- Breeding programmes should be designed for the generation of mono sex populations,
- MT dosage should be limited to a maximum of 50-60 mg MT/kg fry feed.
- Tilapia treated with MT should be reared for at least five months to ensure that the hormone residue remains at zero.
- Precaution measure such as use of latex gloves and a protective face mask should be used when preparing or administering MT to avoid contact.
- Avoid direct discharges of wastewater from the hatchery where MT is used into the environment.
- Hatcheries using MT should use Bio filter followed by gravel and sand filter, plus a shallow vegetated pond to retain and filter wastewater for several days before releasing into the general environment.

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