International Journal of Fisheries and Aquatic Research
ISSN: 2456-7248; Impact Factor: RJIF 5.44
Received: 25-01-2019; Accepted: 20-02-2019
www.fishjournals.com
Volume 4; Issue 2; April 2019; Page No. 42-49

Some methods of monosex tilapia production: A review

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Abstract

Tilapia culture has been growing over the past decades as an excellent source of high-quality protein. The ability to produce monosex is one of the most important factors for the commercialisation and efficient propagation of tilapia fish species, due to influences on reproduction, growth and product quality. In the culture of tilapia seed various issues come into play tilapia when stocked in pond multiply excessively resulting in unhealthy competition for food and natural resources leading to a large number of stunted growths and a large number of mini fish that is not economically viable. Due to sexual diamophisim, the sex of tilapia fish seed produced is skewed towards masculine fish. This paper reviews some methods of monosex tilapia production techniques and their results in form of; periodic harvesting of fries and fingerlings, monosex culture, biological control and sterilization.

Keywords: monosex production, biological control, tilapia, sterilization

1. Introduction

Scientists began their research by focusing on Nile Tilapia because of its ability to breed and produce new generations rapidly, its tolerance for shallow and turbid waters, its high level of disease resistance and its flexibility for culture under many different farming systems (Yosef, 2009; Soto-Zarazúa *et al.*, 2010) [64, 50]. The major drawback of pond culture is the high level of uncontrolled reproduction that may occur in grow-out ponds. Monosex culture is one of the basic methods of controlling Tilapia populations that have been carried out in some countries for aquaculture purposes. This technique includes manual separation of sexes, environmental manipulation, hybridization, hormone augmentation (sex reversal) and genetic manipulation methods such as androgenesis, gynogenesis, polyploidy and transgenesis. Males are preferred because they grow almost twice as fast as females, which may be caused by a sex-specific physiological growth capacity, female mouth-brooding or the more aggressive feeding behavior of males. Expected survival for all-male culture is 90% or greater.

Monosex fish has the ability to tolerate sever environmental conditions including temperature, salinity low dissolve oxygen, greater uniformity of size is achieved at harvest because none of the fish is wasting energy in gonadal development (Ng and Wang, 2011). All male fish is also known for their better flesh quality resistance to prevailing deceases the process of developing gonad spawning and caring come with stress this normal predisposed mixed culture fish to diseases and reduce affect the quality of their skin (Megbowo, 2013; Beardmore *et al.*,, 2001) [6, 38].

A disadvantage of male monosex culture is that fingerlings have to be grown until it is possible to distinguish the female and male juveniles (at least up to 50 g). The density for male monosex culture varies from 10,000 to 50,000/ha or more, at proper feeding rates. Densities of around 10,000/ha allow the fish to grow rapidly without the need for supplemental aeration. About six months is required to produce 500 +g fish from 50 g fingerlings, with a growth rate of 2.5 g/day (Fortes, 2005) [20].

This review examine the problems faced by culturist that farm tilapia and the various ways in which researchers and farmers have being trying to produce monosex tilapia at the optimal level despite the production challenges that is mainly due to the characteristic of fish and production problem based on limited technology and skilled man power among other things.

2. Methods of controlling tilapia population

There are basically four (4) methods used for controlling tilapia populations that have been carried out in some countries for aquaculture and to some extent, eradication purposes. In a number of these countries, tilapia is considered an economic resource and as an important food source for the people. The various methods and/or techniques that follow are:

(1) Periodic harvesting of fry and fingerlings;

(2) Monosex culture of which single-sex fish are obtained through: manual separation of sexes, environmental manipulation for sex determination, hybridization, hormone augmentation and genetic manipulation methods (e.g. androgenesis, gynogenesis, polyploidy and transgenesis);

(3) Biological control

Application of these methods in specific countries has been done with certain degree of success.

(4) Sterilization

2.1 Periodic Harvesting of Tilapia Fry and Fingerlings

Pond culture is still the most popular method of growing tilapia. A mixed-sex population of tilapia is stocked in ponds and the normal pond culture procedures are carried out. However, instead of waiting to obtain the total yield at one harvest, collection of fry and fingerlings is done periodically every one to three weeks. One advantage of this method is that the fish are able to utilize natural foods. However, there is a need for a management scheme in order to maximize tilapia production. The extensive systems (using only organic or inorganic fertilizers) and the semi-intensive systems (using high-protein feed, aeration and water exchange).

2.2 Monosex Culture

Monosex culture may resolve the problem of uncontrolled reproduction in rearing ponds. Rakocy and McGinty (1989) [37] reported the following account relevant to the all-male tilapia culture as follows:

Male monosex culture permits the use of longer culture periods, higher stocking rates and fingerlings of any age. Expected survival for all-male culture is 90 percent or greater. A disadvantage of male monosex culture is that female fingerlings are discarded. The percentage of females mistakenly included in a population of mostly male tilapia affects the maximum attainable size of the original stock in grow-out phase. The stocking rate for male monosex culture varies from 4,000 to 20,000/acre (≈10,000 to 50,000/ha.).

2.2.1 Manual Sorting

Manual sexing, which entails elimination of females based on sexual dimorphism observed in the urogenital papilla, is simple but is time consuming, requires qualified personnel and usually results in 3-10% errors (Hickling, 1963) [26]. This is the process of separating males from females by visual inspection of the external urogenital pores, often with the aid of dye applied to the papillae. Secondary sex characteristics may also be used to help distinguish sex. This requires skilled personnel and trained labor. It necessitates relatively large-size fingerlings (50-80 g) but errors can easily be made even by skilled workers and only about 90% efficient. The sexing of small tilapias although feasible is tedious, time-consuming and often results in a loss of 40-50 percent of the fingerling production due to the discarding of females. This is difficult for large ponds in as much as large numbers of fish are needed and the process is slow. (Hickling, 1963) [26]. Manual sexing is commonly used by producers.

Reliability of sexing depends on the skill of the workers, the species to be sorted and its size. Experienced workers can reliably sex 15-gram fingerling *T. hornorum* and *T. mossambica,* 30-gram *T. nilotica,* and 50-gram *T. aurea.*

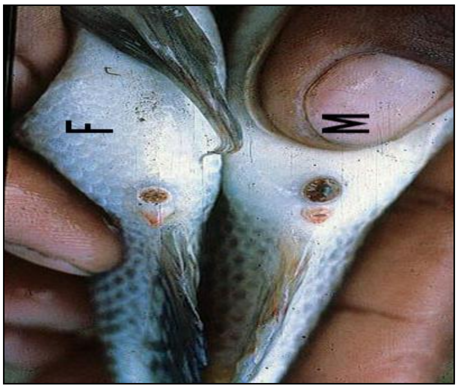

Fig 1: F (Female fish) M (Male fish)

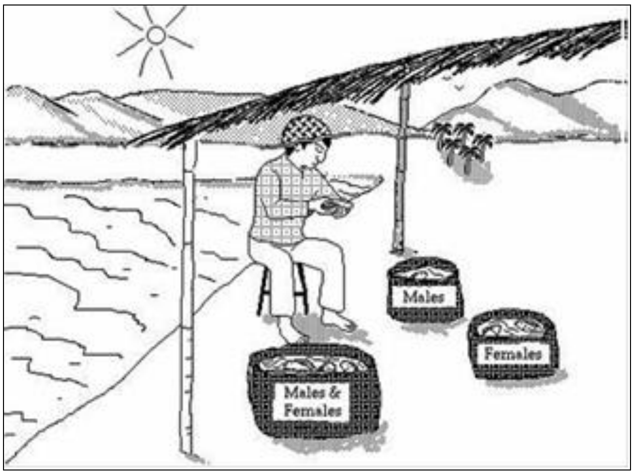

Fig 2: A farmer examining and sorting tilapia by sex.

2.2.2 Environmental Manipulation for Sex Determination

Tilapia is a thermo-sensitive species and its male to female ratio increases with temperature and/or ovarian differentiation is induced by low temperatures. Fish show particularities in their temperature sex determination patterns since monosex populations are generally not produced at extreme temperatures, suggesting the existence of strong temperature genotype interactions. Temperature treatments must be applied at a critical sensitive period, relatively similar to the hormone sensitive period.

Molecular mechanisms of thermo-sensitivity could be addressed in Tilapia species (example *Oreochromis niloticus*), where aromatase gene expression is down regulated by masculinising temperature treatments. Furthermore, in Tilapia, the gene expression of 11β-hydroxylase (a key enzyme involved in the synthesis of 11-oxygenated androgens) does not appear to be affected by temperature treatments (Baroiller and D´Cotta, 2001) [2].

Meanwhile, a strong effect of temperature on sex differentiation has been demonstrated in various Tilapia species and in a hybrid (Baroiller *et al.*,, 1995, Baroiller and Clota, 1998; Desprez and Mélard, 1998; Wang and Tsai, 2000) [15, 60].

2.2.3 Hybridization

Hybridisation is the process of combining different varieties of organism to create a hybrid the crossing of interspecific strains to produce hybrids and the mating of genetically differentiated individuals or groups which may involve crosses within a species (also known as line crossing or strain crossing) or crosses between separate species (Bartley *et al.*,, 2000) [5].

Louis (2002) who stated that hybridization as a method to control tilapia population by producing single-sex fish, obliges fish farmers to either produce or buy F1 hybrids or maintain and control “pure” tilapia parental stocks. However, it does not allow the combination of the characteristics of parental species in any other proportion than a 50/50 ratio. This is not always optimal and it always gives very heterogeneous populations. Production of F1 hybrids that are essentially related to the production of monosex varieties has been the passion in tilapia.

Hybridization takes advantage of qualitative variances to improve genetics in Tilapia by crossing two closely related but distinct subspecies of fish. It is reported that a good number of the crosses carried out to produce monosex fish were made from a combination of the pure breed of the mouth brooding tilapia; this crosses results in producing fish whose sex orientation skewed towards all male. A number of dedicated crosses were made specifically to produce all male fish this include; male *Oreochromis aureus*×female *Oreochromis niloticus* (Wohlfarth, 1994) [64], male *O. hornorum*×female *O. mossambicus* (Hickling, 1960) [26], male *O. mossambicus*×female *O. aureus* (Pierce, 1980) [43] and male *O. mossambicus*×female *O. spilurus niger* (Pruginnin, 1967). If the sex determination system is different, the hybridization between a female homogametic and a male homogametic produces only male offspring (Wohlfarth and Hulata, 1991; Trombka and Avtalion, 1993) [61, 56]. Wohlfarth and Hulata (1991) [61] suggests that the genetic mechanism of sex determination in Tilapias is analogous to that in platy fish, depending on variation in both sex chromosome and autosomally carried factors.

There are more than 100 tilapia species but the most prominent for aquaculture are the Nile tilapia (*O. niloticus*), the Mozambique tilapia (*O. mossambicus*), and the blue tilapia (*O. aureus*) *O. niloticus* or one of its subspecies are commonly preferred in tropical freshwater while *O. aurea* has increased cold tolerance so it is grown in subtropical freshwater. Table 1 shows some of the crosses that lead to all-male progeny and their best results reported. Among the major constraints in producing hybrids are: maintaining the purity of brood stocks, limited fecundity of parent fish which restricts fry production and difficulty in producing sufficient number of hybrid fry due to spawning incompatibilities between the parent species. In as much as not all crosses produce 100% males, the hybrids may still be subjected to manual separation of sexes or hormone augmentation. Some advantages of hybridization are that it saves time, space and feeds, but it is not a perfect solution (Fortes, 2005) [20].

Some other crosses produce fishes that have their sexuality skewed towards all male fish but were not up to the targeted 100% results ranges from 20-98%.

Due to the need to maintain pure line stocks to satisfy these requirements, special hatchery facilities and skilled labor are required. Thus, production of hybrid fingerlings expensive.

Table 1: Hybridization of some tilapia species and proportion of male progeny produced

Crosses (♂ x ♀)	Males (%)	References
O. aureus x O. niloticus (Ugadan strain)	96-100	Pruginin, 1967
O.hornorum x O. spilurus	100	Hulata *et al.*,, 1983
O. aureus x O. vulcani	98-100	Pruginin, 1975
O. macrochir x O. niloticus	100	Wohlfarth *et al.*,, 1994
O.urolepsis hormorum x O. nigra	98-100	Wohlfarth *et al.*,, 1994
O.urolepsis hormorum x O. vulcani	98-100	Majumdar *et al.*,, 1983
O. macrochir x O. mossambucus	100	Majumdar *et al.*,, 1983
O.hornorum x O. niloticus	100	Wohlfarth *et al.*,, 1994
O.urolepsis hormorum x O. niloticus	100	Wohlfarth *et al.*,, 1990
O. aureus x O. niloticus (Stirling strain)	100	Marengoni *et al.*,, 1998
O. aureus x O. mossambicus	100	Beadmore *et al.*,, 2001
O.hornorum x O. mossambicus	100	Hickling, 1960

2.2.4 Sex Reversal or Hormone Augmentation

This is a technique which has been adopted with some degree of success. The principle behind this method lies on the fact that at the stage when the tilapia larvae are said to be sexually undifferentiated (right after hatching up to about 2 weeks or up to the swim-up stage) the extent of the androgen (male hormone) and the estrogen (female hormone) present in a fish is equal thus, augmenting one of the hormones that is originally present in the fish will direct the fish to either male or female depending upon the hormone introduced. Accordingly, if the tilapia larvae are fed with feeds that are incorporated with male hormone (e.g. 17α-methyltestosterone), the fish will develop into phenotypic male (physically appears and functions as male but possesses the female genotype (XX); in the same way, if a female hormone is mixed with the feed that is taken by the fish, then the fish will be directed to phenotypic female (physically appears and functions as female but possesses the male genotype (XY). Feeding the larvae with hormone-treated diet, e.g., 17α-methyltestosterone or estrogen between the second and sixth week after hatching has been observed to have produced high percentage of males and females, respectively. Different steroids have been used over the years to induce sex reversal even if 17α methyltestosterone is the most common (Pandian and Varadaraj, 1990) [41] for *Oreochromis mossambicus*; 17α-ethynyltestosterone (Shelton *et al.*,, 1981) [48] with *O. aureus*; 17α-methylan-drostendiol (Varadaraj and Pandian

(1987) [41] with *O. mossambicus*; mibolerone (Torrans *et al.*,, 1988) with *O. aureus*; norethisterone acetate (Pandian and Varadaraj,1990) [41] with *O. mossambicus*; fluoxymesterone with *O. niloticus* (Phelps *et al.*,, 1992) [42]; trenbolone acetate with *O. aureus* (Galvez *et al.*,, 1996) [21].

Some constraints in the use of hormones in fish is that the presence of hormone residue in adult fish has not yet been studied thus its effect on the consumers is not yet known. Hormones may also be difficult to obtain in some countries and hatchery facilities and skilled labor are required.

Table 2: List of successful use of different type of hormones in producing monosex tilapia

Species	Hormone	Duration	Male (%)	References
Oreochromis niloticus	Fadrozole	30 days	92.5-96	Kwon *et al.*,, (2000)
O. niloticus	17 α-ethynyltestosterone	25-28 days	91-99.4	Afonso *et al.*, (2001)
O. niloticus	17 α methyldihydrotestosterone	21 days	99	Guerrero and Guerror (1988) [48]
O. niloticus	17 α methyldihydrotestosterone	4 h	100	Wassermann and Afonso (200
O. mossambicus	17 α-ethynyltestosterone	18 days	100	Guerrero (1975) [35]
O. mossambicus	17 α-methyltestosterone	42 days	100	Hines and Watts (1995
O. aureus	17 α-ethynyltestosterone	25-28 days	83-97	Melard *et al.*, (1994)

This method can be performed by oral administration of feed incorporated with androgen and eggs or fry immersion in different concentrations of the male hormone.

Hormones may also be difficult to obtain in some countries and hatchery facilities and skilled labour is required (Fortes, 2005) [20]. Sex reversal by oral administration of feed incorporated with methyl testosterone is probably the most effective and practical method for the production of all male Tilapia,

Sex reversal by the immersion technique is achieved by immersing the eggs in different concentrations of 17-αmethyl testosterone exposed for different times. The mechanism of action of the immersion technique is that the hormone is absorbed through passive diffusion across the lipid membrane of the eggs. During the embryonic development, gonadal differentiation can be affected by the administration of steroid sex hormone (Jobling, 1995) [30] in the holding water. Strussman and Nakamura (2003) [53] pointed out that the mechanism of action of exogenous steroids during sex differentiation is not sufficiently clear. Cagauan *et al.*, (2004) [8] evaluated sex reversal of Nile Tilapia *O. niloticus* by immersing the eggs in different concentrations of 17-αmethyl testosterone.

Highest percent male of 91% was attained at 800 μg L-1hormone concentration at 96-h immersion time comparable with the 88 to 89% in 400 to 600 μg L-1hormone concentration at the same immersion time. Sex reversal by egg immersion may lessen the duration of treatment and lower the cost of hormone used relative to the traditional technique of sex reversal by oral administration.

However, this technique presents conflicting results possibly due to the rapid early development that limits the window of opportunity (Contreras-Sánchez, 2001) [10] and these results are lower than those obtained with the immersion of fry (Gale *et al.*,, 1996; Fitzpatrick *et al.*,, 1999; López *et al.*,, 2007) [21, 19] and with the use of feed hormone (Manosrioi *et al.*,, 2004; Jiménez and Arredondo, 2000; Torres and Marquez, 2006) [29, 11].

2.2.5 Genetic Sex Determination

Adopting viable traditional selection methods can improve commercially desirable strains in tilapia. Applying novel DNA-based techniques (e.g., MAS) in genetic stock improvement schemes may consequently give faster results in enhancing tilapia production. The realization of the need to improve the tilapia stocks brought about the creation of the GIFT (Genetically Improved Farmed Tilapia). This started in 1988 when direct transfers of Nile tilapia to the Philippines from Egypt, Ghana, Kenya and Senegal were made. The tilapias were held at the National Freshwater Fishery Training and Research Center/Bureau of Fisheries and Aquatic Resources (NFFTRC/BFAR), Muñoz, Nueva Ecija. These served as the founder stock. From these stocks, production of mixed base population (synthetic breed) was initiated and run for at least one generation of selection. The tilapia that were produced from these are called the GIFT tilapia and it is claimed that they grow at an average of 60% faster than the other breeds.

(Androgenesis, gynogenesis, polyploidy and transgenesis). Genetic improvement in tilapia has been achieved through the exploitation of qualitative variances (e.g., selective breeding, hybridization and crossbreeding and genetic manipulation (e.g., sex control chromosome manipulation and transgenesis). Two examples of successful selection programs in tilapia are the ICLARM-coordinated Genetically Improved Farmed Tilapia (GIFT) Project and the Freshwater Aquaculture Center (FAC)–Central Luzon State University (CLSU) Project which produced the YY Super males. The GIFT and the YY Super Males produced through YY male technology are not considered genetically modified organisms. The GIFT Project reported a 70% increase in growth rate over 7 generations of combined selection in a synthetic Nile tilapia strain developed from newly introduced African and domesticated Asian stocks. The FAC/CLSU Project reported a cumulative response of 18.4% after 16 generations of within family selection using a Philippine strain. Recently, traditional selective breeding methods have been enhanced by advanced DNA based technologies. Molecular markers have been used to identify quantitative trait loci (QTLs) that can enable selection for desirable traits in marker-assisted selection (MAS) programs. Successful genetic manipulation methods, (i.e., gynogenesis, androgenesis, polyploidy and transgenesis) their potential as well as risks to commercial aquaculture have been reported.

To avoid the deterioration in quality of tilapia culture stocks, farmers are advised to choose their cultured strains carefully. The following basic management guidelines should be adopted:

1. Keep high effective population size,
2. Avoid unconscious selection
3. Transfer stocks between hatcheries, and

4. Maintain stock/strain purity.

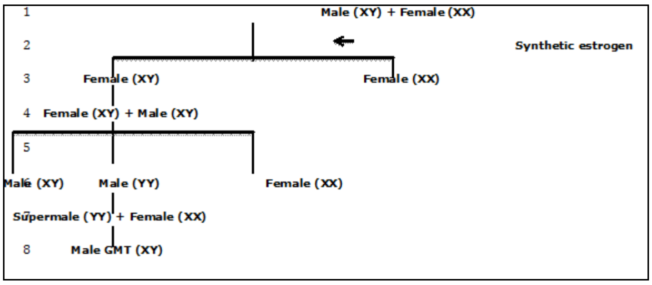

Fig 3

Schematic diagram for producing super male (YY) and all male tilapia (XY) (Tave, 1993).

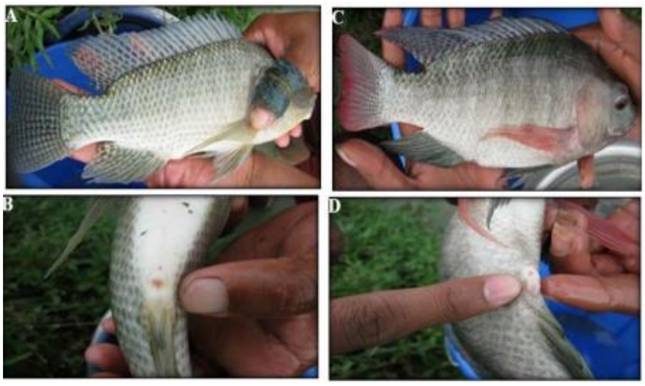

Fig 4: Sex identification of the brood fish: A. GIFT female fish, B. Genital papilla of female GIFT, C. GIFT male fish and D. Genital papilla of male GIFT

Table 3: Distinction between female and male GIFT

s	Female GIFT	Male GIFT
Body Shape	Elongated	Deep bodied
Body colour	Less attractive than male	More attractive than female
Colour of dorsal and caudal fin margin	Grayish	Bright red
Size of genetal papilla	Smaller to male	Larger than female

2.3 Biological control

This is a means of controlling certain fish population by stocking predacious fish as fingerlings or adults in a pond. This concept and method was developed by Dr. Homer W. Swingle, recognized as the Father of Aquaculture in the United States of America who hailed from Auburn University in Alabama. It needs an effective predatory fish that can control excessive reproduction. This method produces at least two different kinds of fish – the predatory fish and the prey species.

In the case of tilapia, large fish must be stocked initially or they will be eaten by the predators. The predators will not only crop down the surplus fry and thereby permit better growth in the *Tilapia* population; they will also contribute to the variety and value of the total fish crop. It has been shown that excessive production of tilapia fry can be overcome by the addition of a predatory species to control the population by feeding on fry and fingerlings. Predator species such as *Lates niloticus*, *Hemichromis fasciatus* and *Clarias lazera* have been used for this purpose (FAO/UNDP Task force on Aquaculture Development and Coordination Programme, 1980)

Using the predator-prey method, one reservoir in Malaca had produced more than 6000 pounds a year of good-sized tilapias weighing between three-quarters of a pound and two pounds each (Hickling, 1963) [26]. In order to keep the predator-prey balance at an advantageous level, there must be frequent stocking. Several species of predatory fishes have also been used for this purpose in other parts of the world. In East Africa the Nile perch (*Lates niloticus*), a fine sporting fish, has been tried; in the Cameron's the black bass and a fish related to the *Tilapia* called *Hemichromis fasciatus* have been stocked in tilapia ponds; in Jamaica the local tarpon (*Megalops cyprinoids*) has served as an effective predator.

In Nigeria, Fagbenro (2000) conducted a quantitative evaluation of the predation efficiency of two hybrid clariid catfishes (*Heterobranchus longifilis* x *Clarias gariepinus)* and (*H. bidorsalis* x *C. gariepinus*) in controlling recruitment of Nile tilapia (*Tilapia niloticus)* while producing market-size fish. This was conducted in small earthen ponds (200 m^2) using three tilapia: hybrid catfish stocking combinations (5:1, 10:1, 20:1). After 180 days the treatments 5:1 and 10:1 showed better production.

In the United States, largemouth bass (*Micropterus salmides*) has been used as the predator for tilapia (*T. aurea*). A forage/predator ratio of 0.7 and 1.4 are suitable for controlling spawn and producing large tilapia and large bass, respectively (Wurts *et al.*, 2001).

Sex-reversed and mixed-sex Nile tilapia were stocked at 2 fish m^2 at sizes of 10.5 to 11.6 g and 7.2 to 8.1 g, respectively. Results show that snakehead were able to completely control Nile tilapia recruitment at all tested predator: stocked-prey ratios, and the best predator: stocked-prey ratio was 1:80. The addition of snakehead into Nile tilapia ponds did not result in significantly greater tilapia growth, but it significantly lowered total net and gross yields of adult plus recruited tilapia.

Various predatory fish species have been used with varying success in combination with different tilapia species depending on their availability. These species include snakehead (*Channa striata* or *Ophiocephalus striatus*) (Pongsuwana, 1956; Chimits, 1957; Tongsanga, 1962; Chen, 1976; Cruz and Shehadeh, 1980; Hopkins *et al.*, 1982; Wee, 1982; Balasuriya, 1988);

However, the difficulty in breeding or obtaining predators of the correct size often resulted in limited application of this population control method (Balarin and Hatton, 1979; Penman and Mc Andrew, 2000) [41].

Biological method to control the forage species requires draining of the pond, sorting out the fish and restocking with the right proportion of predator and prey. The technique is a sophisticated one, with plenty of room for error. Another drawback of this strategy is that often, it is difficult to get adequate numbers of predator fingerlings (Hickling, 1963) [26].

2.4 Sterilization

In Nigeria, Ekanem and Okoronkwo (2003) [16] tried pawpaw (*Carica papaya*) seed as fertility control agent on male Nile tilapia. Mature male tilapia with mean weight 40 g were treated for 30 days with 4.9 g/kg/day (low dose) and a 9.8 g/kg/day (high dose) of ground pawpaw seeds incorporated into their feed. In order to determine the effect of the treatments, a control treatment was added using fish of similar sizes fed with feed that did not contain pawpaw seed. No spawning occurred in any of the replicates in the high dose treatments during the 30-day treatment period. Fish in the control experiment spawned two weeks and five weeks after while fish in the low dose treatment spawned three weeks after the treatment was discontinued.

Pawpaw seeds contain active ingredients such as caricacin, an enzyme carpasemine, a plant growth inhibitor, and oleanolic glycoside, the last of which had been found to cause sterility in male rats (Das, 1980) [13]. Other means to carry out sterilization program is the use of irradiation to cause sterility to the target fish.

There are about 70 species of tilapias, most of them native to Western rivers of Africa (Anon 1984). Of these, nine species are used in aquaculture worldwide (FAO, 2002) [18]. However, tilapia production is concentrated mainly on Nile tilapia (*O. niloticus*), Mozambique tilapia (*O. mossambicus*) and Blue tilapia (*O. aureus*). Of these three species *O. niloticus* has for many decades been responsible for the significant increase in global tilapia production from freshwater aquaculture and accounted for about 83% of total tilapias produced worldwide (FAO, 2002) [18].

3. Conclusion

In Tilapia culture, the desired goal efficiency rate for each technique to produce male Tilapia is between 98 and 100%. The technique employed in the majority of the developing countries is sex reversal because of its easy employment and high rate of success. Among the different techniques used for sex reversal (oral administration, egg immersion and fry immersion) the one that shows the best results is the fry immersion, with a higher success rate and without the risk to employees due to contact with the hormone during the preparation of the feed. Some other advantages of these techniques are: it takes less period of time, less water quality and quantity is needed, less hormone consumption and no influences from fish feeding behavior.

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