

Review Article

<https://doi.org/10.20546/ijcmas.2021.1001.069>

## A Review on the Production and Culture Techniques of Monosex Tilapia

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### ABSTRACT

#### Keywords

Tilapia,  
*Oreochromis niloticus*, Monosex tilapia, Monosex culture, Monosex

#### Article Info

Accepted:  
08 December 2020  
Available Online:  
10 January 2021

Tilapia ranks second among the most cultured fish globally. It is also the most important farmed non-cyprinid fish accounting for over 20% of the global aquaculture production (6.6 million tonnes). To make up for anticipated deficits or losses in wild harvest, the tilapia industry is retorting, not only in its former strongholds throughout Southeast Asia, but also in the Americas and Africa. The candidate aquacultured species of the tilapiine family is the Nile tilapia (*Oreochromis niloticus*), which is known for its superior growth rates and attainment of large market sizes over a wide range of environmental parameters. Its disease resistance and ability to efficiently flex its feeding habits also make it an ideal species for culture. The increased production in tilapia has been recognized on extensive research efforts, with the most significant gains achieved by utilizing monosex production techniques. Monosex tilapia is a new technological product in this aquacultural era.

### Introduction

Capture fisheries production has reached a stagnant stage and is no longer considered capable of sustaining the supply of fisheries products for the growing global demand (Subasinghe *et al.*, 2009). Tilapia has the potential to play a crucial role in the fight against food insecurity and malnutrition (FAO, 2012; 2017; El-Kassas *et al.*, 2020) and has been the only cichlid (Order: Perciformes) maintaining stable market prices across the globe (Wang and Lu, 2016).

Tilapia is one of the most consumed farmed fishes in the world and the most important cultured non-cyprinid fish globally, with a total production of 6.6 million tonnes in 2019, most of which came from countries outside of their natural habitat in Africa and the Levant (Prabu *et al.*, 2019; El-Kassas *et al.*, 2020). It is hardy, environment-friendly and has good resistance to diseases; they are reasonably priced and easy even for small fishers to grow (El-Sayed, 2019). In addition to this, the growing health awareness among people regarding protein-rich diet also enhanced the

demand for tilapia since it contains essential vitamins and omega-3 fatty acids (Karim *et al.*, 2017; El-Sayed, 2019). Moreover, the developments in genetic technology, selective breeding and increasing government initiatives to support aquaculture business are also stimulating the growth of the tilapia market (Prabu *et al.*, 2019). The bulk (80%) of the production comes from a single species, the Nile tilapia (*Oreochromis niloticus*) (Prabu *et al.*, 2019; Mengistu *et al.*, 2020; El-Kassas *et al.*, 2020). There are about 70 species of tilapia amongst which 10 species find its use in aquaculture worldwide (El-Sayed, 2019). Tilapia are primarily herbivorous and detritivorous feeders. However, the presence of flexible and opportunistic feeding behaviour has made them an ideal species for aquaculture (Karim *et al.*, 2017). The development of sex-reversal techniques using hormones in the 1970s led to the production of male monosex populations to be raised to uniform, marketable sizes (Thongprajukaew and Rodjaroen, 2020). In monosex culture, males are preferred because they have a faster growth rate than females (Chávez-García *et al.*, 2020). It serves as an important source of income throughout the world (Sosa *et al.*, 2005). The monosex male population of tilapia are well recognized for increased production potential and low management requirements (El-Sayed, 2019). Over the ages, tilapia has become the shining star of aquaculture and popularly known by different names like ‘aquatic chicken’, ‘fish of the 1990s’ and ‘food fish of the 21st century’ (Costa-Pierce and Riedel, 2000; Ramnarine and Singh, 2005).

### **History of monosex tilapia and its initial production**

The exhibition of sub-optimal growth and variable sizes were the frequent constraints of mixed-sex populations of tilapia commercial culture before 1970s (Noakes and Balon,

1982; Fuentes-Silva *et al.*, 2012). The initial attempts to produce all-male fry in order to circumvent the foresaid problem began in the 1970s. Although it looked promising, no suitable technology for reliably producing all-male tilapia at a commercially viable scale emerged until the mid-1980s. Development of hapa-based culture allowed for the assortment of tilapia eggs and yolk-sac larvae of a uniform age and proved to be the key in ensuring consistently high (~99%) levels of male fish following the application for 21 days of feed augmented with 17- $\alpha$  methyltestosterone (Gale *et al.*, 1996; Popma and Lovshin, 1996). This breakthrough befell as a result of research instigated by the Asian Institute for Technology (AIT) in 1984 as a part of an EU funded project on septage-fed aquaculture systems. After late 1990’s, the number of monosex hatcheries flourished as commercial demand for sex-reversed fry increased and knowledge of the necessary hatchery management techniques, once confined mostly to researchers associated with the early development of the technology, became more accessible. The ability to produce monosex tilapia has revolutionised the profile of the species’ production and consumption.

### **Monosex tilapia production techniques**

The major drawback of tilapia pond culture is the high level of unrestrained reproduction that may occur in grow-out ponds (Little and Hulata, 2000; Felix *et al.*, 2019). Monosex culture is one of the methods of restraining uncontrolled tilapia populations (Green *et al.*, 1997; Little and Hulata, 2000; Wang *et al.*, 2019). Monosex production techniques include the labour-intensive hand-to-hand separation of sexes, environmental alterations, hybridization, hormonal augmentation (sex reversal) and laboratory genetic manipulation methods such as androgenesis, gynogenesis, polyploidy and transgenesis (Felix *et al.*,

2019). None of these methods is consistently 100% effective, and thus an amalgamation of methods is suggested. Males 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 behaviour of males (Kembenya, 2020). Expected survival rate of monosex culture is proved to be above 90% (Abo-Al-Ela, 2018). 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. Male tilapia production has an economic importance. The increase in employment in the sector in view of the world population growth is a crucial source of income and livelihood for hundreds of millions of people around the world (Soto-Zarazúa *et al.*, 2011). It could play a pivotal role in providing food security for the general population as an excellent source of high-quality protein (El-Sayed, 2019).

### **Manual sorting**

Some species of the genus tilapia can be easily sorted into males and females based on their secondary sexual characteristics like colour or the structure of the urogenital papilla (Felix *et al.*, 2019). A second check has to be made when the fish have grown larger and distinctive sex-coloration is more discernible.

The manual sorting of small tilapias based on their sex, although feasible, is tiresome and not entirely trustworthy (Prabu *et al.*, 2019). Additionally, it is stressful for the fish. Human accuracy varies between 80% and 90% entailing for errors in the range of 3-10% (Felix *et al.*, 2019). For this reason, this method is rarely used (Penmann and McAndrew, 2000). This technique may be useful in small populations like backyard aquaculture but unfit for commercial purposes.

### **Environmental temperature manipulation**

Tilapia is a thermo-sensitive species (Prabu *et al.*, 2019). The male to female proportion changes with temperature and/or ovarian differentiation (Mengistu *et al.*, 2020). Temperature treatments must be made at critical periods of time. Molecular mechanisms of thermo-sensitivity could be addressed in tilapia species (*O. niloticus*), where aromatase gene expression is down-regulated by masculinising temperature treatments (Baroiller *et al.*, 2009). Baroiller *et al.*, (1995) demonstrated that tilapias were sensitive to temperature during the period of sex differentiation and there is a possibility to masculinise XX progenies (100% females) with elevated temperatures (above 32°C). High temperatures could capably masculinise some offspring if started around 10 days post fertilization (Baroiller *et al.*, 2009). However, if a treatment was made for a 10-day period beginning at 7 days post fertilization, it had no consequences on sex ratios (Baroiller *et al.*, 1995). Unfortunately most of these studies regarding temperature sensitivity are hindered because the sex determination mechanisms of most of these species are less-studied (Baroiller *et al.*, 2009).

### **Hybridization**

Hybridization aims at the qualitative variances to improve genetics by crossing two closely related but distinct subspecies of fish. Hybrids from various *Oreochromis* species are listed in the Table 1. A super male Tilapia (YY) can be attained by feminizing genotypic males (XY) with estrogens and then breeding them with normal males (XY), which leads to three different possible progenies: females (XX), males (XY) and super males (YY). This strategy has restrictions, mostly, not being 100% effective, which implies the possibility of other sex determining factors (Pham and Little, 1998; Francis and Esa,

2016). The major limitations in producing hybrids are maintenance and purity of brooders, inadequate fecundity and difficulty in producing sufficient hybrid fry due to spawning incompatibilities between the parent species.

### **Sex reversal or hormonal augmentation by feeding**

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 and probably the most popularly (commercially) used technique (Beardmore *et al.*, 2001; Belton and Little, 2008; Celik *et al.*, 2011; Mehrim *et al.*, 2019; Snake *et al.*, 2020). 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 and the estrogen present in a fish is equal (Karim *et al.*, 2017). 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 feed incorporated with male hormone viz., 17 $\alpha$ -methyltestosterone (MT), the fish will develop into phenotypic male physically and function as male but possess the female genotype (XX) (Beardmore *et al.*, 2001). The list of hormones that have been researched and worked upon on various tilapiine species are mentioned in Table 2. Sex reversed “male” reaches similar average weights as genetically male tilapia (Mair *et al.*, 1995) and it does not require that a portion of the production be discarded (Phelps and Popma, 2000). The presence of hormone residue in adult fish has not yet been studied, thus its effect on consumers is not yet known (Suseno *et al.*, 2020). Hormones may be challenging to obtain in some places and

skilled labour with good hatchery facilities are required (Phelps and Popma, 2000). Sex reversal by oral administration of feed (incorporated with 17 $\alpha$ -MT) is the most effective and practical method for the production of all-male tilapia till date (Celik *et al.*, 2011; Snake *et al.*, 2020). The dose when administered orally differs between 30-60 mg/kg of feed for total of 25-30 days depending on the intended outcome (Little *et al.*, 2003). It is suggested that the use of 30 mg/kg of 17 $\alpha$ -ET for 18 days will approximately result in production of 98% of male monosex fish. The use of 60 mg/kg of feed will produce 100% male in *O. mossambicus* (Francis and Esa, 2016). The 17 $\alpha$ -MT will give 98% and 85% at 30 and 60 mg/kg feed. This is an indication that the administration of more than the recommended dose will not add any value to the stock but serve a waste of available resources (Phelps and Popma, 2000; Sayed *et al.*, 2018). However, the technique has minor limitations. Uniform age fish should be used at the first feeding stage to ensure high reversal rate. Moreover, widespread use of vast quantities of androgens in hatcheries may pose a health risk to workers (Mlalila *et al.*, 2015; Francis and Esa, 2016; Sayed *et al.*, 2018; Suseno *et al.*, 2020). This technique has achieved successful results up to 100% and feed with the male hormone is commercially available or can be prepared (Mlalila *et al.*, 2015).

### **Hormonal augmentation using immersion methods**

Another alternative of the oral feeding is the use of live bait that has been brought up in a synthetic environment enriched with androgens [17 $\alpha$ -MT and 17 $\alpha$ -ET] (Sayed *et al.*, 2018). This technique has been used with Nile Tilapia fry and has obtained levels of masculinisation up to 99% (Phelps and Popma, 2000; Syarifuddin *et al.*, 2019).

**Table.1** A list of different hybrids of *Oreochromis* spp. and their probability of male progeny

Male	Female	Male (%)
<i>O. niloticus</i>	<i>O. aureus</i>	75-95
<i>O. aureus</i>	<i>O. niloticus</i>	50-100
<i>O. mossambicus</i>	<i>O. aureus</i>	89
<i>O. hornorum</i>	<i>O. mossambicus</i>	100
<i>O. macrochir</i>	<i>O. mossambicus</i>	100
<i>O. macrochir</i>	<i>O. niloticus</i>	100

(Source: Francis and Esa, 2016)

**Table.2** A list of different reported hormones that have been utilized for production of monosex tilapia

Species	Hormone	Duration	Male (%)
<i>O. niloticus</i>	Fadrozole	30 days	92.5 – 96
<i>O. niloticus</i>	17 $\alpha$ -ET*	25-28 days	91 – 99.4
<i>O. niloticus</i>	17 $\alpha$ -MT*	21 days	99
<i>O. niloticus</i>	17 $\alpha$ -methyl dihydrotestosterone	4 hours	100
<i>O. mossambicus</i>	17 $\alpha$ -ET	18 days	100
<i>O. mossambicus</i>	17 $\alpha$ -MT	18 days	98
<i>O. aureus</i>	17 $\alpha$ -MT	42 days	100
<i>O. aureus</i>	17 $\alpha$ -ET	25-28 days	83 – 97

(Source: Francis and Esa, 2016; Mukherjee et al., 2018)

\* MT: methyltestosterone; ET: ethynyltestosterone

**Table.3** Feeding chart of monosex tilapia in respect to their biomass

Average bodyweight of fish (grams)	Daily feeding rate	Times a day
20-25	8-10 %	3-4
50-100	6-8 %	3-4
100-200	5-6 %	3
>200	1.5-4 %	3

(Source: Francis and Esa, 2016)

(Note: The feeding chart provided is based on the works of the authors mentioned above. The daily feeding rates and doses can be altered in compliance with the changing temperature and other environmental conditions)

Sex reversal by the immersion technique is achieved by immersing the eggs in different concentrations of 17 $\alpha$ -MT exposed for different period of time. The mechanism of action of the immersion technique is that the hormone is absorbed by passive diffusion across the egg membrane. During the embryonic development, gonadal

differentiation can be affected by the administration of steroid sex hormone (Francis and Esa, 2016; Syarifuddin *et al.*, 2019) in the holding water. About 91% of male population was attained when *O. niloticus* eggs were immersed in 17 $\alpha$ -MT (Cagauan *et al.*, 2004) However, this technique presents conflicting results possibly

due to the rapid early development that limits the window of opportunity (Phelps and Popma, 2000) and these results are lower than those obtained with the oral administration (Francis and Esa, 2016)

### **Monosex tilapia culture**

Feed preparation plays a pivotal role in the culture of monosex tilapia (Wangpen, 1996; Rahman *et al.*, 2019). The feed is prepared by an alcohol evaporation method (Wangpen, 1996; Francis and Esa, 2016). Ordinary fish ration (filler) is mixed at (40 to 60%) with a high protein fish meal substitute of roughly 60% protein, which is used as base. The hormone,  $17\alpha$ -MT is dissolved in a solvent (methanol or ethanol) at a rate of 60-70 mg of hormone/L solvent. The prepared mixture is added to the feed at a rate of 60-70 mg/kg feed, hand-mixed thoroughly and allowed to air dry. This is done 3 to 4 days prior usage. This mixture should not be stored for more than 4 days (Chakraborty and Baneerjee, 2009; Chakraborty *et al.*, 2011). Brood ponds should have a firm bottom and good drainage systems (Macintosh and Little, 1995; Chakraborty *et al.*, 2011). Brooders can be fed at 1-3% body weight/day using supplementary feeds (26-35% crude protein). Brood ponds and its stocks should be maintained separately and care must be taken to prevent inbreeding. Re-stocking should be done every 2-3 months. Nursery ponds encounter high mortality during culture days solely due to extreme stocking densities (Guerrero III, 1986; Tsadik and Bart, 2007; Chakraborty *et al.*, 2011).

Nursery stocks should be handled with proper care to reduce stress and mortality. Nursery ponds demand good cleaning practices using fish toxicants (rotenone) and bleach. In the grow-out phase when the growth rate stagnates, it is advised that the stock has reached its marketable size (El-Nahal *et al.*,

2019). Monosex tilapia takes about six months to produce a good market size [500 grams] (Fortes, 2005; Chakraborty *et al.*, 2011). Like any other culture, monosex tilapia doesn't pose serious headaches to farmers. Monosex tilapia is raised through two steps in two ponds viz., nursing and stocking pond. This type of farming system can maximize production. Just like normal tilapia culture, the management of nursing pond and grow out ponds for monosex are quite the same. Feeding with highly protein enriched (about 35%) feed at the rate of 10-15% of the body weight showed good results (Bhujel, 2000). Feeding can be done 3-4 times a day. When the monosex attains a weight of approximately 20-30 grams (40-60 days of culture) they can be transferred to the stocking pond. Care must be taken to reduce stress. Application of good quality organic manure can lead to exponential growth of natural feed and hence the use of supplementary feed gets limited. After the monosex attains a weight of 100 grams each, feeding can be reduced to 5% of body weight. The feeding chart of monosex tilapia is shown in Table 3.

The use of dugout pond is one of the primitive systems used in the production of monosex tilapia seeds (Trong *et al.*, 2013). Culture in hapas is more popular when compared to dugout ponds whereas culture in concrete tanks has received extensive popularity from various monosex fishers recently (Bentsen *et al.*, 1998; Bhujel, 2000; Ghosal *et al.*, 2015; Sarker *et al.*, 2017). Despite all this information available on the production protocols of larval rearing, hatchability and the absorption of yolk sac still remains unique to different geographical locations (Bhujel, 2000). Production is a biological process that must remain unchanged. The major problem of insufficient quality tilapia seeds must be addressed.

### **Tank culture**

Fry (21-28 days post hatching) are stocked for culture from brood ponds after being sorted using a 2.5 mm mesh. Those passing through the mesh are used for sex-reversal and range from approximately 7-11 mm in length. They are stocked in tanks initially where they are chemically treated for diseases and parasites, which they may have assimilated (Shevgoor *et al.*, 1994; Faruk *et al.*, 2019; Razzak *et al.*, 2019). They are then moved to the culture tanks at high stocking densities (5000-6000 per cubic metre). The biologically filtered water is made to recirculate inside the culture tanks at a temperature of 23-26°C. Feeding is done at a rate of 20% body weight and 4 times daily (Chakraborty *et al.*, 2011). The sex-reversal cycle takes approximately 28-30 days and the feeding rate is gradually reduced to 10% body weight at the near end of the cycle (Thongprajukaew and Rodjaroen, 2020). Weekly sampling of the fry is a necessity and this facilitates the check on feeding rates. Ectoparasitic infestations should be carefully monitored with the use of proper prophylactics. During the treatment period, the growth rate is slow due to high stocking densities and maintenance of low treatment temperatures (Faruk *et al.*, 2019). On completion of the treatment, fry are stocked in separate nursery ponds for rapid growth prior to their stocking in production ponds.

### **Cage culture**

Cage monosex culture has developed recent popularity in India (Chakraborty *et al.*, 2007; Ghosal *et al.*, 2015; Sarker *et al.*, 2017; Balkhande, 2019). Fry (21-28 days post hatching) are passed through a 3.1 mm mesh to remove fry larger than 14 mm (Moniruzzaman *et al.*, 2015). The smaller fry are stocked in cages of mesh size 1.5 mm at densities of approximately 2000-3000 per square metre. The fry are fed on the hormone

augmented feed 4 times daily starting at 20% body weight at the initial phase of the treatment cycle and reducing to 10% at completion. The fry in the cages show more significant growth than those in 'clear' water (Kembanya, 2020). The fry are then removed from the cages at the cycle end and stocked in nursery ponds where they are fed on a normal diet. The possible disadvantage of this technique is the loss of thousands of "partially sex-reversed fry" during removal of nets or cages (Kembanya, 2020).

### **Harvesting**

Harvesting of monosex tilapia requires the use of a seine and implements two methods: partial harvesting and drain-down (total harvesting (Shevgoor *et al.*, 1994; Meyer *et al.*, 1997; Sarker *et al.*, 2017). Partial harvesting involves pulling a seine to remove the larger marketable fish and leaving the smaller ones to attain marketable sizes whereas a total harvest is achieved by draining the whole pond and removing all the stocked fish. This is usually done with several seine hauls. Typically, the period between a partial harvest and a total harvest is approximately two weeks. Fish should be harvested in the early hours of the day or at evening to prevent undue stress. Care should also be taken to keep them fresh and transfer them as quickly as possible with good handling practices.

In conclusion the monosex tilapia farming has taken an important role in the fish farming business throughout the world (Sunny *et al.*, 2019). Monosex tilapia has great demand and value in the local as well as the international market. A profitable culture of monosex tilapia depends on two important factors, viz., good farm management practices and proper stocking (Lorenzen, 2000; Lovshin *et al.*, 2000; Atul, 2019). On the other hand, monetary investment is quite low when

compared to other cyprinid and non-cyprinid fishes (Atul, 2019). As a result, monosex tilapia farming rate is growing day by day and have attracted small local fishers. Their ability to grow at extensive stocking densities, good feed intake, extensive growth rates, survivability and high disease resistance make them an excellent species for aquaculture (Sunny *et al.*, 2019). Their ability to grow along Indian major carps (IMCs) in different culture systems also makes them flexible (Ghosal *et al.*, 2015). Their opportunistic feeding behaviour also interests fishers. However, the main hindrance to the commercial market is the hormonal augmentation which may pose threats to the consumers as a residue after culture. Extensive studies are needed and maybe one day, monosex tilapia may become a candidate species in aquaculture.

### **Funding**

The authors declare there is no source of funding.

### **Ethical statement**

The authors declare there are no ethical conflicts in regard with this article.

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#### **How to cite this article:**

Avishek Bardhan, S. K. Sau, Sanjib Khatua, Madhabendu Bera and Paul, B. N. 2021. A Review on the Production and Culture Techniques of Monosex Tilapia. *Int.J.Curr.Microbiol.App.Sci.* 10(01): 565-577. doi: <https://doi.org/10.20546/ijcmas.2021.1001.069>