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(Article begins on next page)

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## Can sterility through triploidy induction make an impact on Tilapia industry?

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**Abstract:** Triploidy induction has been opted as an effective management tool in tilapia fish culture in the past. At present scenario, the production of tilapia monosex population through hormone treatment has become an important tool for aquaculturists to avoid unwanted reproduction and to produce single-sex individuals with larger growth potential. However, there is always the risk of public reacting badly to what they perceive as an unnecessary use of hormones in their food. Therefore a robust and reliable solution to overcome hurdles faced by tilapia aquaculture industry like advanced sexual maturation, unwanted and uncontrolled reproduction is always in demand. This article will discuss the efficacy of sterilization in tilapia using triploidy induction technique and its effect on altering the sex ratio and thereby how much this technique could revive the tilapia farming in the future.

**Key Words:** Tilapia, Sterility, Triploidy, Monosex, Reproduction

### Introduction

Tilapia (genus *Tilapia* and *Oreochromis* of family Cichlidae), a fish endemic to Africa, was once a merely neglected fish because little was known about its usefulness. Later realization of its aquaculture potential led this African hero (Nile tilapia) to be one of the most widely

distributed fish in the world, since 1930. Tilapias are now being cultured all over the world and in fact they are more common in Asia than in their African homeland (Costa-Pierce and Rakocy, 1997). In recent years, great attention has been given to tilapia farming and it has emerged as

one of the most important and internationally-traded fish (Altun *et al.*, 2006). At the present scenario, tilapia farming is a billion dollar industry (\$2.5 billion) that generates more than 2 million metric tons of farmed tilapia each year. In 2007 the production of farmed tilapia reached approximately 2.5 million tons (FAO, 2009). Tilapia is now the second highest farm-raised food fish worldwide next to carp (Fitzsimmons, 2000; Watanabe *et al.*, 2002).

A large volume of information through scientific research has already been gathered for tilapia. Even though tilapias are alien to many countries, they have received as much attention in the tropics as salmonids in the temperate areas. The contribution of tilapia to the protein diet of human population in many areas of the world has prompted the International development agencies to name it as "Aquatic Chicken". Popularity and acceptability of this versatile group of fishes among the world population in the early 1990's has prompted them to be named as "fish of the 1990's" (Costa-Pierce and Rakocy, 2000). But recently they have been considered as "Food Fish of the 21<sup>st</sup> Century" (Ramnarine, 2005). However, tilapias are unusual among tropical fishes in having been relatively well investigated (Leveque, 2002; Pradeep *et al.*, 2011a).

Tilapias have been introduced to different parts of the world to serve various needs including the biological control of aquatic weeds and insects, as bait fish for some capture

fisheries, as food fish in aquaculture systems, as aquarium species and to augment capture fisheries (Canonico *et al.*, 2005). Since then, they have become established in nearly every water body of the territories in which they are cultured or otherwise present. Now they are probably the most widely distributed group of exotic fish worldwide (Courtenay, 1997; Costa-Pierce, 2003).

Though, the tilapia introduction to various countries has been a great success over the past two decades, there also exist controversies and concern regarding the possible environmental and biodiversity damage caused by introduction of tilapias. This topic has been under several decades of debate among the ecologists and environmentalists (Canonico *et al.*, 2005; Pullin *et al.*, 1997). Though aquaculture supremacy of tilapias cannot be questioned, in reality their wide environmental tolerance and adaptability make them a potentially invasive fish in natural ecosystems. In fact, it should be kept in mind that all tilapias, either being cultured or studied, are potentially invasive species (Pullin *et al.*, 1997; Casal, 2006). Moreover, the unscientific approaches in introductions of various species of tilapias into the range of closely related ones have often led to inadvertent hybridization (Levine, 2000).

### **Triploidization in tilapia**

In terms of reproduction, tilapias are paradox. Although uncontrolled reproduction

might sound like a favorable situation, however, most often it can turn out to be big burden for farmers (Pradeep *et al.*, 2011b). Uncontrollable reproduction may result in production of a large number of young ones in the culture conditions. This ultimately leads to stoutness or reduced growth in bigger fishes as the adults need to compete with younger ones for limited resources covering both space and food. However in the past, several small scale farmers considered the prolific breeding activity of tilapia to be one of the most important traits of this species. It facilitated them to avoid repeated purchase of "seeds" from the hatcheries and also contributed to its promotion and distribution for rural development purposes throughout the tropics. But from an aquaculture perspective, reproduction in the culture environment before reaching market size is not an ideal solution. From this perspective tilapia present some challenges to the fish culturist (Phelps and Popma, 2000).

Sterilization has been proposed as the best possible solution to solve the problems concerned with tilapia production (Pradeep *et al.*, 2011c). The production of reproductively sterile fishes have considerable potential in aquaculture and is considered to be one of the best farm management practices in aquaculture because these fishes may use their metabolism to increase their protein content as edible muscle biomass, rather than seasonally shifting to the production of sperm and eggs (Galli, 2002). This strategy offers a way of preventing fish from

becoming environmental pests on escape, whether or not they are transgenic (Maclean, 2003). In the present aquaculture scenario, there is no doubt that tilapia might be the single most appropriate candidate species for large-scale production of functionally sterile seeds (Lutz, 2001). Unlike other sterilization techniques, much of the genetic manipulation studies in tilapia have been concentrated in the production of sterile triploid offspring with an aim to counteract the problem of precocious sexual maturity and unwanted reproduction in culture (Mair, 1993).

The first attempt made on chromosome set manipulation in blue tilapia, *Oreochromis aureus* by Valenti (1975) using temperature-shocks, paved the way for future investigations on polyploidy in tilapia. Following this attempt triploidy induction has successfully been executed through the induced retention of the second polar body in eggs fertilized with viable spermatozoa in three commercially important strains, namely *O. niloticus*, *O. aureus* and *O. mossambicus*. In all these species a 100% triploidy yield was optimized by using cold-shock, heat-shock and pressure-shock treatments by Chourrout and Itskovich (1983), Penman *et al.* (1987), Don and Avtalion (1988) and Hussain *et al.* (1991). The best optimized treatment protocol for triploidy induction in various commercially important strains of tilapia is summarized in Tab. 1.

Information so far regarding the growth of

Table 1: The best treatment protocol for triploidy induction in various strains of tilapia

Species	Induction method	Shock optima			Triploid% at treatment optima <sup>2</sup>	Percent survival <sup>3</sup>	Ref.
		Level	Duration (min)	Shock initiation time <sup>1</sup>			
<u>Nile tilapia</u> <i>O. niloticus</i>	Heat	41 °C	3.5	5	100	70	1
	Cold	9 °C	30	7	100	67	
	Pressure	8000 psi	2	9	100	85	
	Heat	40-41 °C	4-5	4-6	100	31.7-48	4
	Cold	13 °C	45	5	85-100	6.1	
	Heat	41 °C	4.5	4	100	-	3
	Heat	40.5 °C	3.5-4	4	100	7	6
	Heat	41.5 °C	3.5-4	3.5	100	9	
	Cold	11 °C	60	0.5-5	100	41-50	
	<u>Blue tilapia</u> <i>O. aureus</i>	Heat	39.5 °C	3.5-4	3	100	60-61
Heat		39.5 °C	3.5-4	3	100	60	6
Cold		11 °C	60	0.5-5	100	57-60	
Heat		41 °C	4	3	100	74	2
Heat		42 °C	3	3	100	60.7	9
Heat		42 °C	3	2.5	100	63	7
<u>Mozambique tilapia</u> <i>O. mossambicus</i>	Heat	42 °C	3	2.5-4.5	100	70-74	8
	Pressure	7500 psi	2-15	2.5	100	40-80	
<u>Red tilapia</u> <i>O. mossambicus</i>	Heat	41 °C	5	4	91.8	68.7	10
X <i>O. niloticus</i>	Cold	9 °C	30	4	98.7	75.8	11

<sup>1</sup> Minutes after fertilization

<sup>2</sup> Percentage of the treated eggs in which the product has been successfully induced and verified

<sup>3</sup> Expressed as surviving hatchlings as percentage of treated eggs

**Ref.** 1: Hussain *et al.*, 1991; 2: Chang and Liao, 1996; 3: Puckhaber and Schwark, 1996; 4: El Gamal *et al.*, 1999; 5: Don and Avtalion, 1986; 6: Don and Avtalion, 1988; 7: Pandian and Varadaraj, 1988; 8: Varadaraj and Pandian, 1988; 9: Byamungu *et al.*, 2001; 10: Pradeep *et al.*, 2010; 11: Pradeep, 2011

triploid tilapia is limited and has always been a subject of contradiction. The growth performances of triploid tilapia in some cases have been reported to be inferior or almost similar as compared to their diploid counterparts (Don and

Avtalion, 1986; Penman *et al.*, 1987; Chang *et al.*, 1993; Mol *et al.*, 1994; Hussain *et al.*, 1995; Puckhaber and Horstgen-Schwark, 1996). In fact, if examined carefully, almost all these studies were performed on growth of triploid

tilapia in laboratory condition. There is a possibility that the culture condition provided for these experiments might have prevented uncontrolled multiplication in diploid groups which could be one of the reasons for similar growth performance among diploids and triploids. It is a well known fact that males of tilapia grows faster than the female (Tessema *et al.*, 2006), but unfortunately in some cases the triploidy induction has influenced the sex ratios in favour of females and this also has resulted in the reduced yield (Mol *et al.*, 1994; Byamungu *et al.*, 2001).

Although, some researchers found similar or adverse growth in triploid tilapia, all of them agreed that the triploidy induction in tilapia is benefited by its sterility. Studies which have opted for a longer duration in field condition have explored the full potential of sterility by triploidy induction (Jeong *et al.*, 1992; Bramick *et al.*, 1995; Focken *et al.*, 2000). All these studies have showed that there was no change in growth difference between triploids and diploids at the age of sexual maturation, but culture days extended above this period has significantly influenced weight gain in favor of triploids. Hence, for comparing the growth performance of diploid and triploid tilapia, it is necessary to consider two different growth phases: before and after sexual maturity. This is because triploids did not exceed growth of control fish up to sexual maturity as observed from early studies (Mair, 1993). Moreover, growth

advantage has been seen to influence the triploid tilapia when manipulation of feeding regimes and rates were tested (Byamungu *et al.*, 2001; Pechsiri and Yakupitiyage, 2005). This reveals the biological and physiological advantage of triploid tilapias over diploid ones. This information could be very beneficial for tilapia aquaculture farmers.

Varadaraj and Pandian (1990) has reported that sterility through triploidy induction has benefited the females of *O. mossambicus*. In their study, triploid females have showed 14% faster growth than the monosex male (sex reversed) and 23% faster than the triploid male. The technique developed by Varadaraj and Pandian (1990) produced 100% triploid females by combining endocrine sex reversal and chromosome manipulation techniques. These authors suggested that all female triploidization could be a possible solution and a beneficial technique for tilapia aquaculture. Another interesting benefit offered by sterility through triploidy induction in tilapia is that the technique decreases the sexual dimorphism. Study by Bramick *et al.* (1995) showed that sexual dimorphism in diploids were far greater ( $25 \pm 5.9\%$ ) than the triploids which displayed only  $8 \pm 3.4\%$ . Therefore, the discarding of females because of inferior growth rates as practiced in traditional tilapia culture would become needless in triploid populations. Furthermore, a recent study on triploidy induction and growth performance in red tilapia by Pradeep *et al.*

(2012) showed very interesting results, where the heat-shock induction of triploidy showed successful skewness of their progeny towards male sex, which ultimately led to enhanced yield. This result is rather encouraging for tilapia industry, since production of sterile triploid males can be considered as a holistic approach to an alternative and foolproof method for other cumbersome techniques such as monosex male production. It could be thus anticipated in the near future, that triploidy induction technique could culminate the use of steroid hormones, which are not encouraged by many aquaculturists.

### Conclusion

The importance of tilapia in aquaculture is obviously unquestionable. Since the increase in demand for fish as a protein source and the decline of world capture fisheries is very evident, no other fish in the near future can hold the status of cheaply and easily available protein source as enjoyed by this common man fish-“*Tilapia*”. Hence, any methods for improving tilapia performance in aquaculture are of fundamental significance for the improvement of the diet, particularly in developing countries.

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### Reference

- ✓ Altun T., Tekelioglu N. and Danabas D. (2006) Tilapia Culture and Its Problems in Turkey. *J. Fish. Aquat. Sci.*, 23(3-4): 473-478.
- ✓ Bramick U., Puckhaber B., Langholz H.J. and Horstgen-Schwark G. (1995) Testing of triploid Tilapia (*Oreochromis niloticus*) under tropical pond conditions. *Aquaculture*, 137: 343-353.
- ✓ Byamungu N., Darras V.M. and Kuhn E.R. (2001) Growth of heat-shock induced triploids of blue tilapia, *Oreochromis aureus*, reared in tanks and in ponds in Eastern Congo: feeding regimes and compensatory growth response of triploid females. *Aquaculture*, 198: 109-122.
- ✓ Canonico G.C., Arthington A., Mccrary J.K. and Thieme M.L. (2005) The effects of introduced tilapias on native biodiversity. *Aquat. Conservat. Mar. Freshwat. Ecosyst.*, 15: 463-483.
- ✓ Casal C.M.V. (2006) Global documentation of fish introductions: the growing crisis and recommendations for action. *Biol. Invasions.*, 8: 3-11.
- ✓ Chang S.L., Chang C.F. and Liao I.C. (1993) Comparative study on the growth and gonadal development of diploid and triploid tilapia, *Oreochromis aureus*. *J. Taiwan. Fish. Res.*, 1(1): 43-49.
- ✓ Chang S.L. and Liao I.C. (1996) Triploidy induced by heat shock in *Oreochromis aureus*. In: Pullin, R.S.V., Lazard, J., Legendre, M., Amon Kothias, J.B. and Pauly, D. (eds) The Third International Symposium on Tilapia in Aquaculture ICLARM Conference Proceeding, 41: 273-279.
- ✓ Chourrout D. and Itskovich J. (1983) Three manipulations permitted by artificial insemination in tilapia: induced diploid gynogenesis, production of all triploid populations and intergeneric hybridization. In: Fishelson, L. and Yaron, Z. (eds) Proceedings of the International Symposium on Tilapia in Aquaculture, Tel Aviv University, Tel Aviv, Israel, 246-255.
- ✓ Costa-Pierce B.A. (2003) Rapid evolution of an established feral tilapia (*Oreochromis* sp.): the need to incorporate invasion science into regulatory structures. *Biol. Invasions.*, 5: 71-84.

- ✓ Costa-Pierce B.A. and Rakocy J.E. (1997) Tilapia aquaculture in the Americas, Vol. 1. World Aquaculture Society, Baston Rouge, Louisiane, United States.
- ✓ Costa-Pierce B.A. and Rakocy J.E. (2000) Tilapia aquaculture in the Americas, World Aquaculture Society, Baston Rouge, Louisiane, United States.
- ✓ Courtenay W.R. (1997) Tilapias as non-indigenous species in the Americas: environmental, regulatory and legal issues. In: Costa-Pierce, B.A. and Rakocy, J.E. (eds) Tilapia Aquaculture in the Americas. World Aquaculture Society: Baton Rouge, LA, 1: 18-33.
- ✓ Don, J. and Avtalion R.R. (1986) The induction of tiploidy in *Oreochromis aureus* by heat shock. Theor. Appl. Genet., 72: 186-192.
- ✓ Don, J. and Avtalion R.R. (1988) Comparative study on the induction of triploidy in tilapias, using cold- and heat-shock techniques. J. Fish Biol., 32: 665-672.
- ✓ El Gamal A.R.A., Davis K.B., Jenkins J.A. and Torrns E.L. (1999) Induction of triploidy and tetraploidy in Nile Tilapia, *Oreochromis niloticus* (L.). J. World Aquacult. Soc., 30(2): 269-275.
- ✓ Fitzsimmons, K. (2000) Future trends of tilapia aquaculture in the Americas. In: Costa-Pierce, B.A. and Rakocy, J.E. (eds) Tilapia Aquaculture in the Americas, Baton Rouge, Louisiana, United States, 2: 252-264.
- ✓ Focken U., Hörstgen-Schwark G., Lückstädt C. and Becker K. (2000) Growth, metabolic rates and body composition of individually reared triploid tilapia (*Oreochromis niloticus*) in comparison to diploid full-sibs. In: Fifth International Symposium on Tilapia in Aquaculture, ISTA V, Sept 3-7, Rio de Janeiro, Brazil, 2: 359-365.
- ✓ Food and Agriculture Organization of the United Nations. FAO yearbook. (2009) Fishery and Aquaculture Statistics.
- ✓ Galli L. (2002) Genetic modification in aquaculture- A review of potential benefits and risks. Bureau of Rural Sciences, Canberra.
- ✓ Hussain M.G., Chatterji A., McAndrew B.J. and Johnstone R. (1991) Triploidy induction in Nile tilapia, *Oreochromis niloticus* L. using pressure, heat and cold shocks. Theor. Appl. Genet., 81: 6-12.
- ✓ Hussain M.G., Rao G.P.S., Humayun N.M., Randall C.F., Penman D.J., Kime D., Bromage N.R., Myers J.M. and McAndrew B.J. (1995) Comparative performance of growth, biochemical composition and endocrine profiles in diploid and triploid tilapia *Oreochromis niloticus* L. Aquaculture, 138: 87-97.
- ✓ Jeong W.G., Hue J.S. and Kim E.O. (1992) Induction of triploidy, gonadal development and growth in the Nile tilapia, *Oreochromis niloticus*. Bull. Nat. Fish. Res. Dev. Agency., 46: 161-171.
- ✓ Leveque C. (2002) Out of Africa: the success story of tilapias. Environ. Biol. Fish., 64: 461-464.
- ✓ Levine J.M. (2000) Species diversity and biological invasions: relating local process to community pattern. Science, 288: 852-854.
- ✓ Lutz C.G. (2001) Practical Genetics for Aquaculture. Fishing News Books, Blackwell Science, Oxford.
- ✓ Maclean N. (2003) Genetically modified fish and their effects on food quality and human health and nutrition. Trends. Food Sci. Tech., 14: 242-252.
- ✓ Mair G.C. (1993) Chromosome-set manipulation in tilapia - techniques, problems and prospects. Aquaculture, 111: 227-244.
- ✓ Mol K., Byamungul N., Cuisset B., Yaron Z., Ofir M., Melard Ch., Castelli M. and Kuhn E.R. (1994) Hormonal profile of growing male and female diploids and triploids of the blue tilapia, *Oreochromis aureus*, reared in intensive culture. Fish. Physiol. Biochem., 13(3): 209-218.
- ✓ Pandian T.J. and Varadaraj K. (1988) Techniques for producing all male and all triploid *Oreochromis mossambicus*, In: Pullin R.S.V., Bhukeswaran T., Tonguthai K. and Maclean J.L. (eds) The Second International Symposium on Tilapia in Aquaculture ICLARM Conference Proceedings 15, Department of Fisheries, Bangkok, Thailand and International Centre for Living Aquatic resources Management, Manila, Philippines, 243-249.
- ✓ Pechsiri J. and Yakupitiyage A. (2005) A comparative study of growth and feed utilization efficiency of sex-reversed diploid and triploid Nile tilapia, *Oreochromis niloticus* L. Aquacult. Res., 36: 45-51.
- ✓ Penman D.J., Skibinski D.O.F. and Beardmore J.A. (1987)

- Survival, growth rate and maturity in triploid tilapia. In: Tiewes K. (ed.) Proceedings of the World Symposium on Selection, Hybridization, and Genetic Engineering in Aquaculture, Vol. II. 27-30, May Bordeaux, France. Heinemann, Berlin, 277-287.
- ✓ Phelps R.P. and Popma T.J. (2000) Sex reversal of tilapia. In: Costa-Pierce, B.A. and Rakocy, J.E. (eds). Tilapia Aquaculture in the Americas. The World Aquaculture Society, Baton Rouge, Louisiana, United States, 2: 34-59.
- ✓ Pradeep P.J. (2011) Production of triploidy in red tilapia [*Oreochromis mossambicus* (Peters, 1852) × *Oreochromis niloticus* (Linnaeus, 1758)]. Ph.D. Thesis. University Malaysia Terengganu, 306.
- ✓ Pradeep P.J., Srijaya T.C., Shahreeza M.S., Mithun S., Anuar H. and Anil A. (2010) Induction of triploidy in red tilapia, *Oreochromis mossambicus* (Peters, 1852) × *Oreochromis niloticus* (Linnaeus, 1758) by heat shock treatment under laboratory conditions. J. Coast. Envir., 1(1): 91-102.
- ✓ Pradeep P.J., Srijaya T.C., Shaharom F. and Chatterji A. (2011a) A brief of tilapia, their classification, distribution and perspective. In: Shaharom, F., Pradeep, P.J. and Anil C.H (eds) Tilapia: Aquaculture techniques and potential, Universiti Malaysia Terengganu publication.:7-17.
- ✓ Pradeep P.J., Srijaya T.C., Shaharom F. and Chatterji A. (2011b) Monosex Tilapia: Culture Applications and Techniques. In: Shaharom, F., Pradeep, P.J. and Anil C.H. (eds). Tilapia: Aquaculture techniques and potential, Universiti Malaysia Terengganu publication.:195-213.
- ✓ Pradeep P.J., Srijaya T.C., Jose D., Papini A., Hassan A. and Chatterji A. (2011c) Identification of diploid and triploid Red Tilapia by using erythrocyte indices. Caryologia, 64(4): 485-492.
- ✓ Pradeep P.J., Srijaya T.C., Papini A. and Chatterji A. (2012) Effects of triploidy induction on growth and masculinization of red tilapia [*Oreochromis mossambicus* (Peters, 1852) × *Oreochromis niloticus* (Linnaeus, 1758)]. Aquaculture, 344-349: 181-187.
- ✓ Puckhaber B. and Horstgen-schwark G. (1996) Growth and gonadal development of triploid tilapia (*Oreochromis niloticus*). In: Pullin R.S.V., Lazard M., Legendre J.B., Amon Kothlas and Pauly D. (eds) The Third International Symposium on Tilapia in Aquaculture, ICLARM Conference Proceeding, 575.
- ✓ Pullin R.S.V., Palomares M.L., Casal C.V., Dey M.M. and Pauly D. (1997) Environmental impacts of tilapias. In: Fitzsimmons K. (ed.) Tilapia aquaculture. Proceedings from the Fourth International Symposium on Tilapia in Aquaculture, Northeast Regional Agriculture Engineering Service (NRAES) Cooperative Extension. Ithaca, New York, 2: 554-570.
- ✓ Ramnarine I.W. (2005) The Farming of Tilapia. <http://www.uwi.tt/uwitoday/2005/march/tilapia.asp>.
- ✓ Tessema M., Müller-Belecke A. and Hörstgen-Schwark G. (2006) Effect of rearing temperatures on the sex ratios of *Oreochromis niloticus* populations. Aquaculture, 258: 270-277.
- ✓ Valenti R.J. (1975) Induced polyploidy in *Tilapia aurea* (Steindachner) by means of temperature shock treatment. J. Fish Biol., 7: 519-528.
- ✓ Varadaraj K. and Pandian T.J. (1988) Induction of triploids in *Oreochromis mossambicus* by thermal, hydrostatic pressure and chemical shocks. Proceed. Aquacult. Internat. Cong. Expo., 531-535.
- ✓ Varadaraj K. and Pandian T.J. (1990) Production of all-female sterile-triploid *Oreochromis mossambicus*. Aquaculture, 84: 117-123.
- ✓ Watanabe W.O., Losordo T.M., Fitzsimmons K. and Hanley F. (2002) Tilapia Production Systems in the Americas: Technological Advances, Trends, and Challenges. Rev. Fish. Sci., 10(3&4): 465-498.