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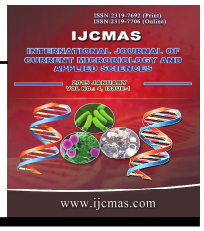

Review Article

Steroidogenic and thermal control of sex in tilapia (*O. niloticus*): A review

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A B S T R A C T

In tilapia production, early sexual maturation and unwanted reproduction have been controlled by different techniques (manual sexing, direct hormonal sex reversal, hybridization or genetic manipulation), practices and their appropriate application. Hormonal and environmental factors in sex differentiation can be exploited for commercial value in aquaculture. This paper reviews male and female tilapia differentiation methods and comparison between current treatments and high temperature treatment for the all male tilapia production. Sex reversal in tilapia is a complex process that involves both genetic sex determination and temperature linked sex determination with interplay of hormones and receptor activity. Genetic variation affects adaptation to temperature as seen in evidence of strain differences and ability to respond to temperature induced sex inversion at three different categories of response. Treatment of tilapia with Methyltestosterone (MT) is still the preferred choice for tilapia masculinization giving the commercial benefits of all male populations in aquaculture. Concerns about the effects of hormone use on the environment as well as tilapia consumers have been allayed.

Introduction

Tilapia attains sexual maturity at about 20-40 g weight, depending on quantity and quality of feed and feeding regimes (Hussain, 2004; de Graf *et al.*, 1999; El-Sayed, 2006). Sexual dimorphism is expressed in the size of the genital papilla with the male genital papilla being larger than that of the females. The male genital papilla has 2 openings (Figure 1): the urogenital opening (excreting urine and

releasing milt) and the anus while the female papilla is flat, shorter and has 3 openings (Figure 1), which are the anus, the urethra and the oviduct (Hussain, 2004). Breeding starts with building and defending of the spawning territory by the males. The female releases eggs while the male releases milt right over the eggs with its genital papilla pressing against the nest. In nature, Nile tilapia (*Oreochromis niloticus*) is a maternal mouth brooder (the female takes the fertilized eggs into her mouth for incubation). Hatching takes place after 70–90 hours of incubation in the mouth at 28 ± 1^{0}C and the female holds the hatched larvae and gives parental care until the swim up stage, which can be between 6–10 days. The weight of first feeding tiny fry of Nile tilapia is about 0.01g and after this stage they are able to take natural or artificial feeds and move easily in the surrounding waters and do not need any parental care (Hussain, 2004; de Graf *et al.*, 1999; El-Sayed, 2006).

In *O. niloticus* embryonic development there are five easily observed developmental stages (Hussain, 2004):

- Morula stage : 6–8 hours after fertilization (haf)
- Pigmentation stage : 45–50 haf
- Hatching stage : 70–90 haf
- Yolk sac absorption stage : 6–10 days after fertilization (daf)
- First feeding stage : 12–14 daf

This paper reviews male and female tilapia differentiation methods and comparison between current treatments and high temperature treatment for the all male tilapia production.

Germ cells development in early gonad

As reviewed by Devlin and Nagahama (2002), fish egg activation and egg fertilization are followed by repeated mitotic cell division to produce a blastula and further cell development, cell inductions, interactions, and differentiation toward the gastrula stage. Fish gonad structure is similar to vertebrate gonads; these have germ cells associated with somatic cells. Germ cells are derived from primordial germ cells which are the pre-sex differentiation state of germ cells and are distinct from somatic cells at early embryonic development (Yoshizaki *et al.*, 2002). According to Nakamura *et al.* (1998), formation of gonad primordial cell during ontogenesis has important effect for gonad sex differentiation. Differentiation of germ cells via meiotic division occurs earlier in female embryos (35 days after hatching) than in male embryos (85 days after hatching) in *Oreochromis niloticus* (Kobayashi, 2010). In Mozambique tilapia, at 3 days after hatching, genital ridges are formed and the germ cells shift from the mesentery into the gonadal regions with somatic cells surrounding them and protruding into the peritoneal wall (Nakamura, 2013).

Two mechanisms have been proposed to explain the process of PGC formation viz-a-viz: the germplasm mechanism and the epigenetic factor mechanism (Yoshizaki *et al.*, 2002). The biochemistry and genetic information transcribed in RNA which regulates this process was hitherto unknown. Recently, information on genes and endocrine control of sexual differentiation has begun to emerge. Li *et al.*, (2011) reported that the transcription factor POD1 is necessary for both onset of sexual development and gonadal development in Nile tilapia. In addition, Sun *et al.*, (2012) reported the strong expression of fibroblast growth factors FGF16 and FGF20b in the ovaries of putative XX female ovaries and

XY sex reversed female ovaries suggesting the role of the FGF9/16/20 growth factors in early oocyte development in female *O. niloticus*. The follicle stimulating hormone receptor (FSHR) has been reported to critically affect the early (3 days post hatching-DPH) differentiation of gonads in the Nile tilapia while Luteinizing hormone and its receptor (LHCGRbb) play a role later on at 25dph hence a limited role in sexual differentiation (Yan *et al.,* 2012).

The somatic cells and PGCs are differentiated in order to form follicles and comprise of oocyte surrounded by inner granulosa and outer thecal layer in ovarian development (Nagahama *et al.,* 1982; Devlin and Nagahama, 2002). Ovarian development is first recognizable with the somatic cells proliferation, oogonia and early oocyte differentiation followed by the formation of cavity. According to Nakamura *et al.* (1998), just after hatching the germ cells in ovaries outnumber those in putative testes. Ovarian differentiation usually occurs earlier than testicular development. In Tilapia, meiotic germ cells have been reported (Kobayashi *et al.,* 2000; Kobayashi and Nagahama, 2009) to appear between 25 to 30 days after hatching in ovaries of fry while their appearance in testicles is usually between 70 to 90 days after hatching Therefore, male differentiation is not easy to detect using testicular morphology since germ cells that are tailored towards testicular development usually remain quiet until after 70 days of larval growth. Spermatogenesis is started at 50–70 days after hatching in two species of tilapia *O. mossambicus* and *O. niloticus* (Nakamura *et al.,* 1998).

Germ cell differentiation

Genetic basis of germ cell differentiation (Figure 2) in tilapia lies in the expression of cyp19a1a gene which creates sexual dimorphic expression even before the onset of morphological sexual differentiation occurs in primordial gonads (Ijiri *et al.,* 2008). Sequel to the formation of the gonadal primordium at 3 dph (days post hatching), the first sign of sexual dimorphism appears after 9 dph as a difference in germ cell numbers between the sexes, followed by the formation of the anlagen of the intratesticular ovarian cavity and efferent duct in XX and XY fry at 20–25 dph, respectively (Kobayashi, 2010).

Early gonads of putative ovaries have more germ cells than those in testes (Kobayashi *et al.,* 2008). In vitro culture of gonads from *O. niloticus* also revealed a similar trend although onset of meiotic division in XX gonads was delayed by eight days as against the 23 dph in vivo while initiation of spermatogenesis was observed after 50dph in XY and meiotic cells appeared 85 dph (Kobayashi, 2010). Hines *et al.* (1999) however reported the presence of gonads in *O. niloticus* at 15 days after fertilization with the appearance of ovaries at 36 days after fertilization and undifferentiated gonads possessing germinal cells in many individuals which they proposed may eventually develop as testes.

Endocrinology of Sex Differentiation

Artificial manipulation to induce sex change using genetic, hormonal or environmental factors is possible in fish hence a high level of flexibility (Pandian and Sheela, 1995). Steroids produced by the gonadal tissues have been proposed to be the factors responsible for onset of sex differentiation in many species of fish including *O. niloticus*. The follicle cells around the oocyte and the leydig cells of the interstitial tissue in the testis are primarily responsible for production of sex steroids in sexually matured fish (Nagahama *et al.,* 1982). Hines

et al. (1999) reported the early endocrine activity of *O. niloticus* using radioimmunoassay as well as in vitro incubation. They reported that fertilized eggs initially have a high level of androgens with fall in concentration as development advanced and gonads emerged. Estradiol levels were found to be low up until 43 days after fertilization and before this time, levels of Testosterone and 11-ketoestosterone increased. Steroid producing cells have been reported to be responsible for the production of steroids that form the ovarian lumen as well as germ cell differentiation (McMillan, 2007). Genetic controls of steroid synthesis that trigger sex differentiation have been attributed to the expression of steroidogenic enzymes. Up regulation of several steroidogenic enzymes prior to initiation of meiosis have been reported including *aldh1a2* in germ cells for XX and XY chromosomal gonads (Dong *et al.,* 2012), *cyp19a1a* which is secreted in the gonads (Yan *et al.,* 2012; Tao *et al.,* 2013). Sex however seems to be a determining factor in the expression of *cyp19a1a* steroidogenic enzyme with early (5 days after hatching) expression in XX larvae and later expression (90 days after hatching) in XY larvae alongside *cyp11b2* (Tao *et al.,* 2013). However, it has been hypothesized that the *DMRT1* gene inhibits *cyp19a1a* expression hence allowing testicular development (Guiguen *et al.,* 2010). Kobayashi *et al.* (2008) reported that *tDMRT1* modulates sex reversal in XX progeny reversed using androgens and ceased completely in XY progeny reversed with estrogen. The exogenous treatment of *O. niloticus* for sex reversal has been linked with the prevalence of both estrogen and androgen receptors in XX progeny gonads at 5 days after hatching and only estrogen receptors in XY progeny gonads (Tao *et al.,* 2013). The *cyp19* gene is responsible for production of aromatase cytochrome P450 which is a terminal enzyme catalyzing the synthesis of estrogen from androgens (Chang *et al.,* 2005). Differentiation into ovaries is assisted by the transcription factor foxl2 which regulates the aromatase promoter required for co-activating aromatase in conjunction with either CAMP or nr5a1 (Guiguen *et al.,* 2010). The effects of sex steroids pertaining to gonadal development and differentiation are only possible if receptors for the steroids are present. The timing of receptor release coincides with steroid release hence a full expression of steroidal effects. In Tilapia, estrogen and estrogenic receptors (ERα, ERβ1 and ERβ2) are present within 5 days after hatching (Ijiri *et al.,* 2008) (Figure 3).

Hormonal (Androgen Hormone) Treatment in Sex Reversal Tilapia

The Nile tilapia is a highly prolific species that attains early sexual maturity hence overpopulation and decreased growth rate (Hussain, 2004; El-Sayed, 2006). The male tilapia attains a greater size at maturity than the female hence it is the sex of choice for aquaculture (Desprez *et al.,* 2003) with greater advantage under monosex cultivation (Beardmore *et al.,* 2001; Hussain, 2004; El-Sayed, 2006). The production of all-male Tilapia therefore has been a subject of research with the use of several androgens being reported. Androgens promote male sex characteristics development as well as anabolic activity that is expressed as increase in body weight due to increased protein biosynthesis (Desprez *et al.,* 2003; Khalil *et al.,* 2011). Oral administration of 17α-Methyl Testosterone (17α-MT) has gained prominence in the tilapia aquaculture industry because it is more convenient and less cumbersome than other methods (Phelps *et al.,* 1996; Beardmore *et al.,* 2001; Macintosh, 2008; El-Greisy and El-Gamal, 2012). Various hormones have been reported to induce masculinization either as

standalone trials or with other methods. Bart (2002) reported the use of trenbolone acetate (TBA) and 17α-Methyldihydrotestosterone (MDHT) in tandem with ultrasound in immersion protocols (1 and 2 hours) with greater success being recorded for TBA combination with ultrasound at a TBA dose of 250 mg L^{-1}. Gale *et al.* (1999) also reported the use of MDHT at a dose of 500 µg l^{-1} for 3 hour immersion treatments of fertilized eggs at 10 and 13 days post fertilization.

Dosage and method of administration as well as duration of administration as reported in research are diverse. The use of 60 mg MT kg^{-1} feed has become popular and it is being used commercially with efficacy being up to 95% (Phelps *et al.*, 1996), however doses range between 30 to 60 mg MT kg^{-1} feed (Boyd, 2004). Ferdous and Ali (2011) recorded 94.28% all male conversion rate using 60 mg MT kg^{-1} of feed while Beaven and Muposhi (2012) obtained 90 % male population using the same dose with crude protein content of feed being 40%. Combination of MT treatments and feed protein levels have been reported to affect tilapia growth although inconsistencies still trail the various dosage being used as expressed in rate of masculinization. Adel *et al.* (2006) also reported a rise in masculinization using 17 α-methyltestosterone (MT) feed (60 mg MT kg^{-1} feed) in combination with protein level of 40 % in feed hence a 100% masculinization efficiency. Similarly, Asad *et al.* (2010) reported the optimization of masculinization (100%) as well as growth using a combination of 70 mg MT kg^{-1} feed and 40% crude protein diet. Phelps *et al.* (1996) reported that doses ranging from 15 through 60 mg MT/kg feed produced 97% masculinization. However various reports show less than this percentage. Ferdous and Ali (2011) reported masculinization rates of between 88.57 and 91.43% for MT doses ranging from 40 mg MT/kg feed to 70 mg MT/kg feed. Also, Das *et al.* (2010) reported between 88.3 % to 96% masculinization with MT doses of 15 mg MT kg^{-1} feed to 60 mg MT kg^{-1} feed. In contrast to this report, Celik *et al.* (2011) reported efficacies between 69.8% and 93.7% for MT doses ranging from 20 mg MT kg^{-1} feed to 60 mg MT kg^{-1} feed. These reports are conflicting and according to Phelps *et al.* (1996), the inability to separate environmental effects may be a key factor but on further investigation, they revealed that treatment environment does not affect the efficacy of MT treatments. The difference in efficacies across various reports is yet to be explained although aspects of genetics and strain differences may need to be investigated. Combinations of hormonal sex reversal studies as well as grow out afterwards have also revealed divergent results. While Asad *et al.* (2010) reported a harvest biomass of 2000.7 kg/ha/year with the combined use of 70 mg MT kg^{-1} feed and a crude protein diet of 40% over 183 days rearing period, Mateen and Ahmed (2007) reported 3342 kg/ha/year using the same hormonal dose and crude protein level in feed with a rearing period of 280 days post masculinization. Usage of MT via immersion protocols on the other hand has not been quite impressive compared to other androgens. Gale *et al.* (1999) reported higher masculinization efficiency for MDHT at 500 µg l^{-1} for two immersion periods at 10 and 13 days post fertilization as against just one immersion at 13 days post fertilization. However, MDHT immersion alongside ultrasound treatment did not perform better than TBA (Bart, 2002). Increasing the dosage of MT administered via feed seems to reduce its efficacy in masculinization. The use of 100 mg MT kg^{-1} of feed produced 79.38% male population of *O. mossambicus* as against 98.09% produced by 75 mg MT kg^{-1} feed

(Marjani *et al.,* 2009). Similarly, Das *et al.* (2010) reported a decline in masculinization from 96% using 60 mg MT kg^{-1} feed to 78.33% using 120 mg MT kg^{-1} feed.

Commercialization of sex reversed tilapia

Prior to the development of the hormone therapy for inducing masculinization, Tilapia aquaculture was riddled with the problem of sub-optimal growth and low size variability (Belton *et al.,* 2009) caused by precocious puberty. The ability to control sex using exogenous hormone therapy is of great commercial value since male tilapia which has the greater economic value in terms of size can be produced. Tilapia aquaculture is popular worldwide with more than 135 countries producing the species (FAO, 2014). In terms of production volume, FAO (2011) estimated that commercial production was 2.5 million tonnes in 2007 with a value of $3.3 billion. In 2008, total production was 3.6 million tones with aquaculture accounting for 78% of this volume (Feidi, 2010). In countries where use of Methyltestosterone (MT) is allowed, such as Thailand, MT plays an important role in the production of tilapia. In Thailand, commercial production of tilapia kick started with the development of hapa system by Asian Institute of Technology (AIT) in the 1980s. This system enabled better control of reproduction hence ease of egg collection, determination of the harvested egg number and frequency of harvesting with the added advantage of sorting of eggs into stages (Little *et al.,* 1997; Bhujel and Arul, 2000; Little *et al.,* 2000; Bhujel *et al.,* 2001; Belton *et al.,* 2009). Commercialization of producing male fish was followed by utilization of androgenic hormone to reverse fish sex. According to Bhujel (2002), 90% of tilapia grow out farms in Thailand use sex reversed Tilapia while 6% culture mix-sex fish. Duration of feeding of MT hormone however varies among research reports (Phelps and Popma, 2000). The feeding period of MT hormone is between 21 AND 35 days (Table 1).

Although the use of MT feed is important, there is need to minimize dosage with the use of a standard dose. Cost as a factor in the production of the feed is also important to the farmer. According to Little *et al.* (1997), the percentage of cost in administering MT hormone feeding are fishmeal (44.5%), alcohol (29.8%), hormone (14.9%), labor (7.8%), vitamin (1.7%) and others (1.2%). However, there is no record of the amount of MT that is used annually worldwide in the production of tilapia (Macintosh, 2008).

Exogenous Hormonal Control of Sex in Farmed Tilapia

Male tilapia can be feminized using exogenous estrogens because estrogen receptors are expressed early enough in their gonads (Paul-Prasanth *et al.,* 2011).Natural testosterone is ineffective as an androgen in fish if administered orally (Brueggemeier, 1986; Phelps *et al.,* 1996) hence the use of synthetic steroid hormones such as 17α-Methyltestosterone with prolonged action either via intramuscular injections or oral administration. Non-steroidal compounds can also be used to disrupt steroid binding hence aromatase which is a catalyst in the biosynthesis of estrogens from androgens can be inhibited to produce all male populations (Piferrer *et al.,* 1994; D'Cotta *et al.,* 2001). Furthermore, compounds that block the binding sites for estrogens also have masculinization effects on female fish. Singh *et al.* (2012) reported a dose of 200 µg l^{-1} of tamoxifen to be effective in masculinization (90%) of *O. niloticus* with 60 days of feeding. Bhandari *et al.* (2006)

reported a reduction in expression of three steroidogenic enzymes: P450 cholesterol side chain cleavage, 3β-hydroxysteroid dehydrogenase, and cytochrome P450 aromatase in the gonads of female tilapia *Oreochromis niloticus* within 15 days of treatment with methyltestosterone (MT at a dose of 50µg g^{-1} diet).

However, the disadvantages of using hormonal sex reversal may include its cost, fear of hormonal residue in the environment, effects on fish farm worker's as well as processor's health and public perception about side effects of hormone used. Khalil *et al.* (2011) said that plasma concentration of the androgen hormone and residual concentration of androgen hormone in the treated fish muscles are not significantly different at the end of treatment (7 months). Macintosh (2008) reported that MT levels in tilapia treated with the hormone reduce by 99% in 100 hours of withdrawal. Discharge of water from tilapia farms with MT treatment protocol should be done via sand filters and biofilters so that discharge concentration is reduced by disintegration.

High temperature treatment in producing more male tilapia

Temperature of the culture environment has been shown to affect sex ratios of some fish, tilapia inclusive. The change in sex of tilapia from female to male increase with high temperature while ovarian differentiation is induced by low temperatures (Baroiller and D'Cotta, 2001). Gonad differentiation of progenies of some fish species can be directed toward testicular differentiation by high temperature treatment applied at 10 days post fertilization (Tessema *et al.*, 2006). Increased water temperature during sex differentiation favours testicular development hence a greater male population with the aid of hormonal activity. D'Cotta *et al.* (2001) reported the reduction of aromatase activity in tilapia at 35^{o}C regardless of sex. The complex sex determining system in *Oreochromis niloticus* was determined by the interactions between a genetic and the temperature influence. In wild populations, sex reversal could be due to the masculinizing effect of temperature when some fry colonized shallow waters at the point of sex differentiation. The ability of a fish species to respond to temperature induced sex reversal must be heritable and traceable to parental stock hence it is an evolutionary trait that involves a V_E (Environmental variance - Temperature) and genotype interaction (V_G) which is revealed in family sex ratios as a result of strong parental effects (Shen and Wang, 2014). Tessema *et al.* (2006), also reported the linkage between temperature treatment and heritability of temperature sex inversion in tilapia with the "Y" gene being responsible for determination of sex inversion in temperature treatments. The effectiveness of temperature treatments in the masculinization of fish has also been traced to increased stress level leading to higher blood cortisol levels and associated masculinization although the mechanism is unclear (Martínez *et al.*, 2014). Bezault *et al.* (2007), confirmed that genotype-environment interactions play a key role in temperature induced sex determination in *O. niloticus.*

Rougeot *et al.* (2008) reported the pre-hatching induction of masculinization during embryonic development via temperature treatment of fertilized eggs with a relatively low success rate compared to dietary administration of hormones as well as temperature treatment post hatching. They reported low survival and masculinization with increasing temperature. Survival is reported to be reduced if masculinizing

temperature increases, (Azaza, 2008). Tessema *et al.*, (2006) reported that masculinization rate did not increase with increasing temperature from 36°C to 38°C but strain response to high temperature treatment differed while Rougeot *et al.* (2008) confirmed that high temperature treatment affects survival more than temperature duration.

Table.1 Effect of various dosages of 17 α-Methyltestosterone (as mg of MT kg-1 of diet), treatment periods, environments, and percent sex inversion of tilapia

Species/ Doses	Days	Environments	% Males	References
O. niloticus				
Control	28	45 L glass aquaria	44.5	Okoko (1996)
MT-3.75		Fed 15% BW (4x/d);	80.0	
MT-7.5		Flow through; 29^{0}C	91.7	
MT-15			98.3	
MT-30			99.3	
MT-60			97.0	
MT-120			71.9	
MT-240			50.7	
MT-480			48.3	
MT-600			55.0	
MT-1200			52.0	
Control	28	Hapa in ponds; 26^{0}C;	51.3	Green and Teichert-Coddington (1994)
MT-60		Fed 20% BW (4x/d)	96.8	
Control	28	Hapa suspended in tanks;	54.7	Phelps *et al.* (1996)
MT-60		Fed 20% BW (4x/d)	97.8	
Control	25	95 L steel tank;	50.4	Tayamen and Shelton (1978)
	35	Continous flow: 23^{0}C	56.9	
	59	Fed 10% BW (4x/d)	54.0	
MT-30	25		99.2	
	35		100.0	
	59		100.0	
MT-60	25		100.0	
	35		100.0	
	59		100.0	
Control	28	115 L steel tank;	44.0	Shepperd (1984)
MT-30		Flow through: 24^0-32^{0}C	98.0	
MT-60		Fed 15% BW (4x/d)	98.0	

Source: Phelps and Popma (2000)

Table.2 Survival rates at the end of the temperature treatment from several references

Strain/ population	Treatment	%Survival rate (mean)	Mini-maxi (%)	Reference
Lake Manzala,	Control (28°C)	95	69-100	Tessema *et al.*(2006)
Egypt	18°C for 20 days starting at 10 dpf	91	67-99	
	36°C for 10 days starting at 10 dpf	95	86-100	
	38°C for 10 days starting at 10 dpf	87	74-100	
Bouyake,	Control (27°C)	96	93-99	Baroiller and Clota, unpub. Data
Ivory Coast	36°C for 30 days starting at 10 dpf	90	87-95	
Lake Volta,	Control (27°C)	92		Bezault *et al.* (2007)
Ghana	36°C for 30 days starting at 10 dpf	95		
Lake Manzala,	Control (27°C)	50	16-72	Rougeot *et al.* (2008)
Egypt	34°C till hatching starting before 12hpf	53	19-72	
	34°C till hatching starting before 12hpf	42	21-66	
	34°C till hatching starting before 12hpf	30	11-57	

Source: Baroiller *et al.* (2009)

Fig.1 (a) The male genital papilla; having 2 openings and (b) The female genital papilla; having 3 openings (Source: Hussain, 2004)

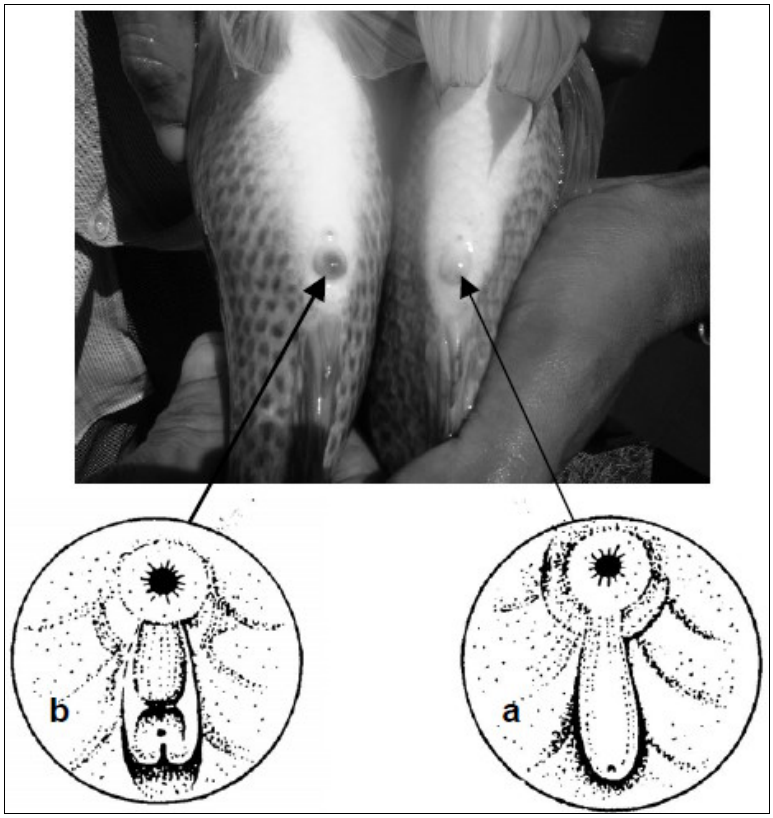
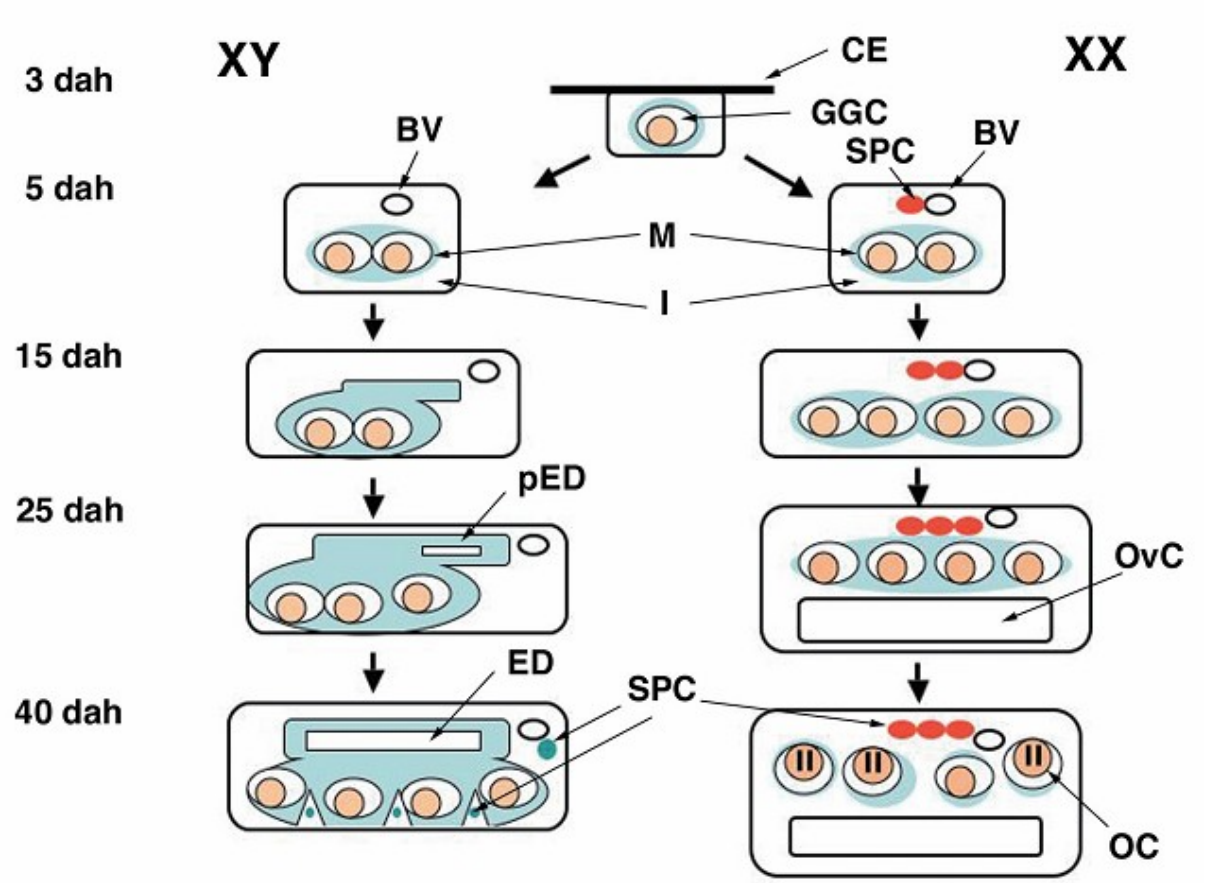
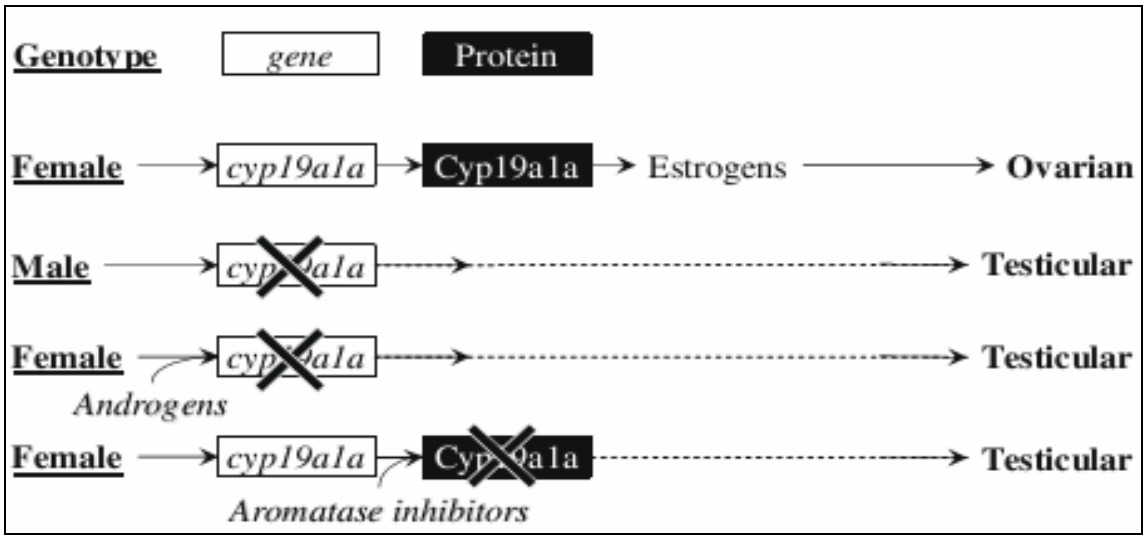


Fig.2 Sexual dimorphism during gonadal differentiation in tilapia (schematic representation of gonadal histogenesis). dah: days after hatching, CE: Coelomic epithelium, GGC : gonial germ cell, SPC: steroid producing cell, BV: Blood vessel, OvC: Ovarian cavity, pED: Analgen of intratesticular efferent duct, Oc: Oocyte, ED: Intratesticular efferent duct, M: Medullary cell, I: Interstitum. (Source: Kobayashi, 2010)

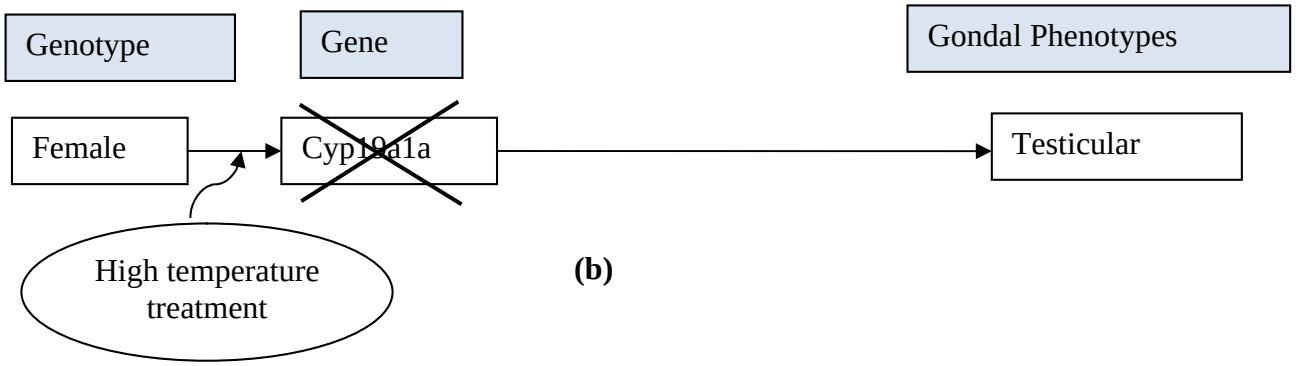


The effect of parental strain and genetics on sex reversal in tilapia was also proven by Wessels and Schwazrk (2007). They showed that line selection of offspring's from parental strains showing high sensitivity to temperature induced sex reversal and those showing low sensitivity was inheritable with 90% masculinization reported for crosses of offspring produced from F_1 generation to produce F_2 generation while the low tolerance group yielded 54% masculinization. Early temperature treatment does not affect sex ratios but sex genotype was found to be of great importance even though fish are classified as low or highly sensitive to thermal treatment (Wessel and Schwazrk, 2007). Variation in survival rate with early temperature treatment (Table 2) is also linked to strains and environment with natural populations of tilapia in Egypt, Ivory Coast and Ghana showing survival rates between 30 to 90% when treated at 34^{o}C to 35^{o}C (Baroiller *et al.*, 2009). The combination of triploidy and masculinization in red tilapia was reported to be effective with a high level of correlation and proportion of males (84.1%) while yolk sac stage showed greater survival (Pradeep *et al.* 2012).

Fig.3 (a) Androgens hormone promotes down-regulation of cyp19a1a gene and aromatase inhibitors blocks Cyp19a1a enzyme activity to derive female to male gonad differentiation (Source: Guiguen *et al.*, 2010), (b) High temperature treatment hormone promotes down-regulation of cyp19a1a gene



(a)



(b)

Temperature control of sex determination

In both gonochoristic and hermaphroditic fishes, sex differentiation and sex inversion can be influenced by environmental factors with temperature being the most important factor for gonochoristic fish and social factors for hermaphroditic fish. Gonochoristic species can be grouped into three categories based on their response to temperature as: greater male percentage at high temperature, more male population at low temperatures and more males at temperature extremes (Ospina-Alvarez and Piferrer, 2008).

Tilapia (*Oreochromis niloticus*) sex is determined by genetic factor (GSD), by temperature (TSD) or by interaction of temperature and genotype. Male gonad differentiation can be achieved by applying high temperature treatment during the critical period of sex differentiation.

Conclusively, sex reversal in tilapia is a

complex process that involves both genetic sex determination and temperature linked sex determination with interplay of hormones and receptor activity. Genetic variation affects adaptation to temperature as seen in evidence of strain differences and ability to respond to temperature induced sex inversion at three different categories of response. Treatment of tilapia with MT is still the preferred choice for tilapia masculinization given the commercial benefits of all male populations in aquaculture. Concerns about the effects of hormone use on the environment as well as tilapia consumers have been allayed.

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