Potential of YY Tilapia Male Technology

The effect on farming, disease resistance, ecosystems and human welfare with Mexico and Ghana as examples

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Summary

For tilapia sex-reversal the deployment of the hormone testosterone is common use with some important unintended side-effects. In this context the potential of YY-technology is positioned.

The effect of release of sex-reversed (SR) tilapia in nature, voluntarily (restocking programs) or not (escapees) on the development of all female Tilapia populations in the ecosystem is neglected. The effect of testosterone on the suppression of the immune system and the effects on disease resistance is explained..

In most of the countries the use of hormones for sex-reversal of consumption fish is normally forbidden but often condoned. This results in no control on the preparation and application. Human welfare is in cause.

Mexico is worldwide an important tilapia producer. All produced tilapia, aquaculture or through restocking programs, is consumed in Mexico. The quality of the fingerlings is very variable.

In Ghana only the use of the local tilapia strain is allowed until today. This strain is very weak and very vulnerable for diseases. Many diseases manifest on this fish like, monogeneans, Columnaris disease, Saprolegnia, Streptocossus spp and Edwardsiella are found. Also the performance of this strain is disappointing.

Introduction

Today, tilapia represents the 4th most important seafood (by weight) in Mexico after sardine, shrimp and tuna, and the 3rd most valuable (Conapesca, 2012). Tilapia is the main product by far from inland fisheries (90% of total outputs) and is the 3rd most important farmed species in Mexico.

During the 1990s Mexico became one of the world's major producers and consumers of tilapia. National production reached a record of 94,279 t in 1996. However, recent official records registered only 77,547 t in 2012 (Figure 1). Most tilapia outputs in 2012 came from fisheries (69%) (Including wild (6%) and restocking (63%) stocks), with aquaculture supplying only 31%. Fisheries has seen a gradual decline in the past 2 decades (going from 93,451 t in 1996 to only 53,791 t in 2012), whereas aquaculture has fast developed only in

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the past 5 years (going from around 4,000 t in 2008 to 23,756 t in 2012). Aquacultures outputs were negligible in less than a decade ago (representing only 1% of Mexico's outputs in 2003).

Despite constant huge efforts and investments in governmental led restocking programs to support the tilapia fisheries in Mexico, most of the fisheries decline was due to lower restocking stocks yields, registering only 49,023 t in 2012. Poor fisheries management and bad fishing practices are mainly to blame to this decline, e.g. overfishing, use of smaller mesh size and no-compliance of closed fishing seasons.

Additionally, the availability of reliable, suitable and good quality fingerlings in Mexico for both, fisheries restocking programs and aquaculture is extremely variable; from well-developed fast growing strains to very poor performing. The large number of hatcheries present, many of them with very little experience, and the large number of tilapia species and strains, have led to mix results amongst farmers. As a result, apart from few well developed mayor players, the tilapia aquaculture industry in Mexico has been characterized by a large number underperforming, small scale and predominantly new to the activity operations, and commonly with short lifespan in their business venture.

In Ghana Tilapia aquaculture is very recent. It is the deployment of cages in Lake Volta that has boost the Tilapia culture.

With 8,520 km2 of inland lakes and rivers, Ghana has a variety of investment opportunities in the inland fisheries subsector. The fish farming subsector of the national economy comprises freshwater fisheries and fish farming or aquaculture.

According to the Ghana News Agency, as cited by vibe.com, the fisheries sector as a whole contributes some 4.5% to 5% of the Gross Domestic Product (GDP) of Ghana over the past couple of years. However, production is still mainly for the national market. Total annual fish production from aquaculture currently stands at over 400,000 tonnes in the last years.

The fresh water fishing industry in Ghana is located mainly along the banks of the Volta Lake, which is the source of about 90% of inland fish production in Ghana. The main fish species that is farmed is tilapia, which the fish farmers do mostly cultivate through cages

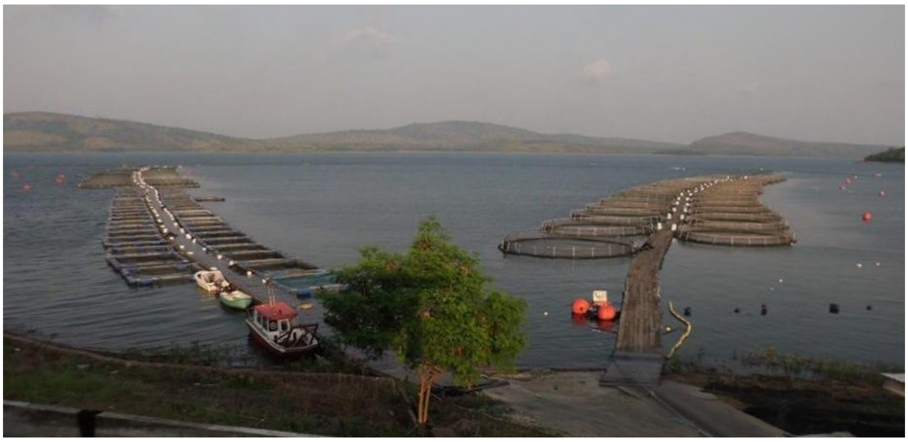

Figure 1. Tilapia cage farm Lake Volta, Ghana

This cage culture utilizes bodies of water such as dams, rivers, lakes, bays and reservoirs. According to the Fisheries Commission, there are about 1,000 fish farmers working on over 2,000 ponds with a total surface area of 350 hectares.

Escapees is daily practice in Lake Volta changing the sexual structure of the natural Tilapia populations and introducing a weak fish in the natural ecosystem.

The availability of reliable, suitable and good quality fingerlings in Ghana for both, fisheries restocking programs and aquaculture is extremely variable. The large number of hatcheries present, many of them with very little experience, and the weak local Tilapia strains, have led to mix results amongst farmers.

Both countries stand for a good example of Tilapia farming with hormonal sex-reversed in many other countries.

Tilapia Fingerling Supply

Tilapia mix-sex population in Mexico and Ghana are employed mainly in some fisheries restocking programs and some small scale and basic aquaculture operations. However the culture of monosex progeny, preferably males in tilapia species, grow faster and to a larger size than females. This has long been recognised as the most effective solution to the widespread problem of early sexual maturation and uncontrolled reproduction in tilapia culture (Mair et al. 1997). To date, this has been achieved either through manual sexing, hybridization, direct hormonal sex reversal (MT-Tilapia; MT=Methyl-Testosterone) or YY male tilapia technology. All with advantages and disadvantages in their application and only hormonal sex reversal has become widely used worldwide, like in Mexico and Ghana.

Nevertheless, a key issue poorly understood and rarely noted and addressed is the potential detrimental effects in gender ratios imbalances of wild populations caused by the release of fertile MT-Tilapia into the wild and their interaction with wild populations, either from purposed-planned restocking governmental programs and/or accidental escapees from aquaculture. This could lead to larger proportions of females in wild populations, with slow growth rates and longer periods to reach market size. Therefore, potentially contributing to the constant size reduction of wild stocks harvested by fishermen.

YY male Tilapia technology has the potential to address this issue whereas providing many other benefits for the environment, the industry (including fisheries and aquaculture) and their ever more demanding domestic and international markets searching for sustainable sources of quality seafood.

Tilapia sex manipulation

Sex determination in *O. niloticus*, while influenced by several genetic and environmental factors, is best described as “predominantly monofactorial”, with an underlying mechanism of male heterogamety (XY) and female homogametry (XX) playing the major role (Mair et al., 1990 & 1997, Scott et al., 1989 and Dunham, R. 2004).

Tilapia male (XY) or female (XX) genotype is established at fertilization, whereas phenotypic sex determination occurs 3-4 weeks after hatching (Shelton et al., 1978). The phenotypic sex can be altered by administration of oestrogens or androgens and by temperature shocks during the critical period of sex determination to produce skewed, all-female or all-male populations. The dosage of artificial hormone is sufficient to overcome the natural hormone or gene product and dictate the sex of the individual (Dunham, R. 2004).

Hormone-treated Sex-reversed Tilapia

One of the most efficacious and widely used androgenic hormone is 17 α – Methyl-Testosterone (MT) (Dunham, 1990a). The standard hormone treatment procedure for tilapias involves adding MT to commercial fry feed, which is then administered to batches of fry of similar age during the short period of their early development when they are most susceptible to the masculinisation effect of this hormone (Macitonch, D. 2008). The outcome of the treatment is the phenotypic masculinization of genetic females, resulting in fertile sex-reversed homogametic males (XX ♂).

Several factors influence the effectiveness of sex reversal in tilapia, including species of fish (Yamazaki, 1983), genetics (Shepperd, 1984; El-Gamal, 1987), type of hormone (Shepperd, 1984), dosage of hormone (Guerrero, 1974; Jo et al., 1988), duration of treatment (Popma, 1987), timing of treatment (Popma, 1987) and operator experience. Resulting in hormone treatment becoming a highly variable technique (Panorama Acuicola, 2012) with unreliable results, many times ranging from 65 to 99% masculinization efficiency.

Nevertheless, XX males have the same capacity to breed with any fertile natural female (also homogametic XX ♀) from either wild populations if released and/or on-farm broodstock as their natural heterogametic male (XY ♂) siblings. The result of this cross-breed is 100% homogametic females (XX ♀) as shown in the graph below (Figure 2):

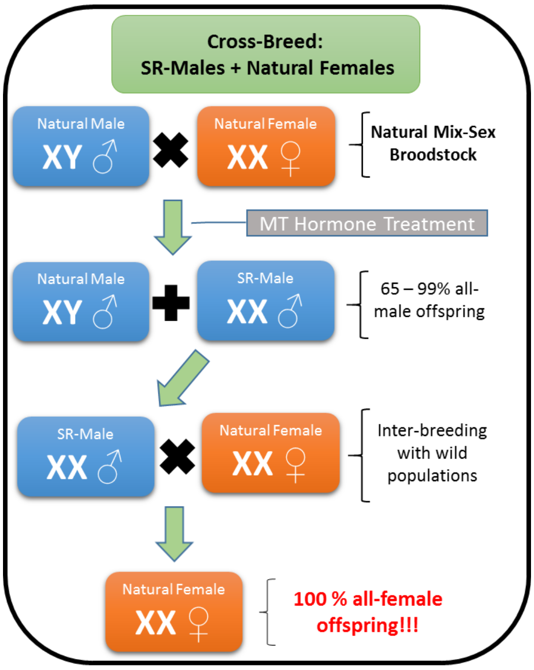

Figure 2. Schematic description of cross-breeding outcome between SR-Males and natural females

Although the extent of MT treatment is unknown, it can be assumed that a high proportion of the tilapias imported into western consumer markets as whole fish, and virtually all fillets, have been MT-treated (Macintosh, D. 2008). The global production of farmed tilapia is projected to rise to 3 million tons by 2010, with the increase in demand being greatest for tilapia fillets (Fitzsimmons, 2008). Hence use of MT is expected to increase in line with this growth in tilapia production. Much of the increased production is expected to come from Nile tilapia farming (FAO, 2006).

Restocking programs employing SR-Tilapia might be an advantage for the first generation stocked, with fast growing males. However, when considering that 50% of SR-Tilapia are natural males (XY ♂) and 50% are SR-Males (XX ♂), the resulting offspring when inter-breeding with wild populations will be 25% natural males (XY ♂) and 75% natural females (XX ♀) (Figure 3).

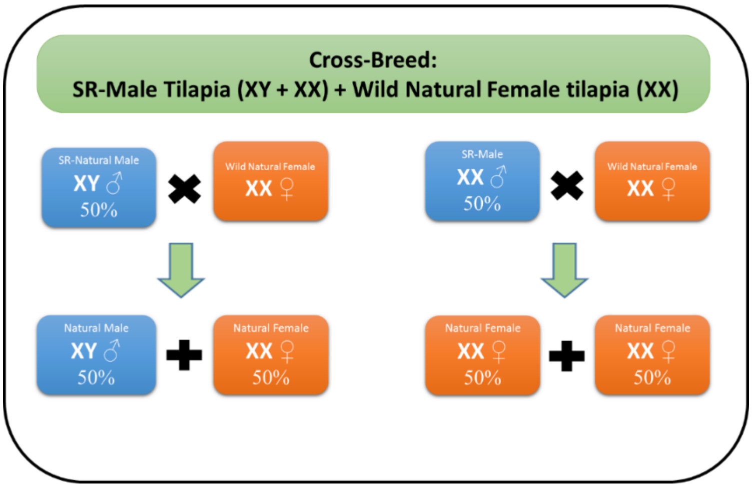

Figure 3. Schematic description of cross-breeding offspring proportion outcome between SR-male tilapia and wild natural females.

Natural Male Tilapia (YY Male Tilapia Technology)

The most successful sex-reversal and breeding programme with the greatest economic impact in tilapia has been the production of all-male XY populations of Nile tilapia from YY males (Dunham, 2004). Mair et al (2001) concluded that "YY male technology" provides a robust and reliable solution to the serious and widespread problem of early sexual maturation, unwanted reproduction and overpopulation in tilapia culture.

YY Male Tilapia Technology is based on the genetic manipulation of sex. This is achieved through a combination of feminization and progeny testing to identify the novel YY genotype (Fig 4), that sires only XY natural male progeny (Herrera et al, 2001) (Fig 5) or Natural Male Tilapia (NMT) (Til-Aqua, pers. comm.). However, the occurrence of occasional females/males can occur from the action of several autosomal sex-modifying genes (Mair et al, 2000, Herrera et al, 2001). Feminization of the YY genotype is a vital step in the development of the YY technology on a large scale (Fig 4), as it makes possible the production of 'YY' males without the need for time consuming progeny testing (Mair et al, 1997).

Feminization (XY females) can be achieved through gynogenesis (UV or γ irradiation) and sex reversal (hormonal or thermal treatment), (Varadaraj et al. 1994, Abucay et al. 1999, Karayucel et al., 2003, Dunhgam, 2004, Lutz, 2006, Phelps, 2006). However, although not always feasible with any strain of tilapia, SR-Thermal treatment has proved to be a more desirable method to produce XY SR-females in large scale operations, as avoids the employment of hormones at any stage of the production process (Eric Bink – Til-Aqua pers. comm.). A characteristic especially important when producing tilapia in countries where the use of hormones is prohibited at any stage of the production process of food products. This in addition to the potential environmental and health related risks that hormone usage can have

(Macintoch, 2006) and the increasing interest of major markets for sustainable and environmentally-friendly food sources.

Progeny testing XY females with normal XY males should spawn 75% males (25% YY males, 50% XY males) and 25% XX females result from the XY - XY mating. Then Male progeny from this mating are then grown for a second generation of progeny testing. When the YY males are test-crossed with normal XX females, 100% XY male progeny result (Mair et al., 2001 and Dunham, 2004).

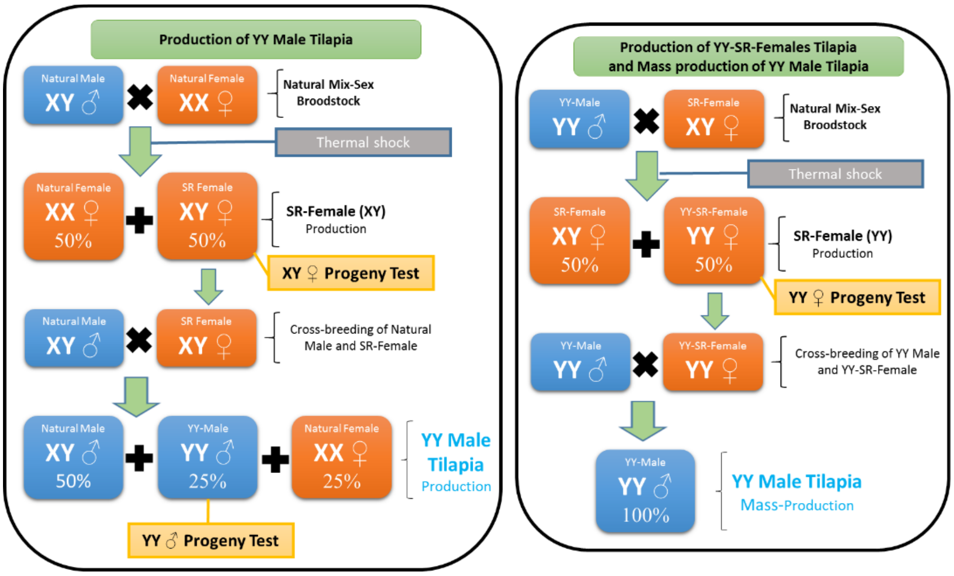

Figure 4. Schematic production of YY male tilapia and its mass production through YY SR female tilapia.

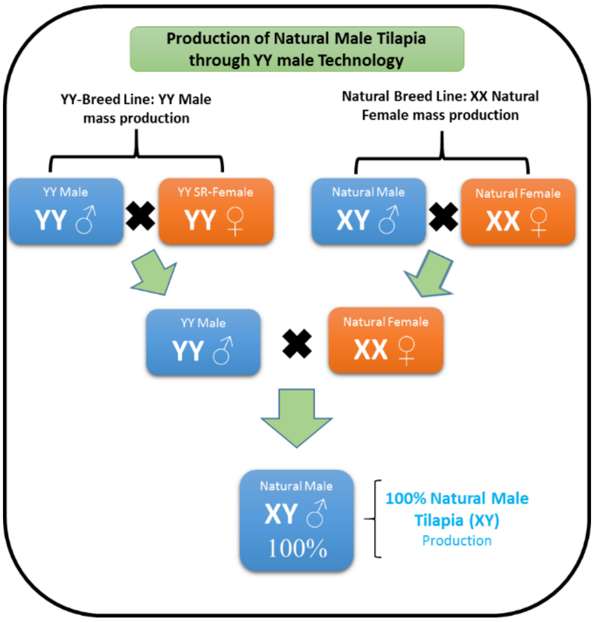

Figure 5. Schematic production of Natural Male Tilapia sired by YY male Tilapia.

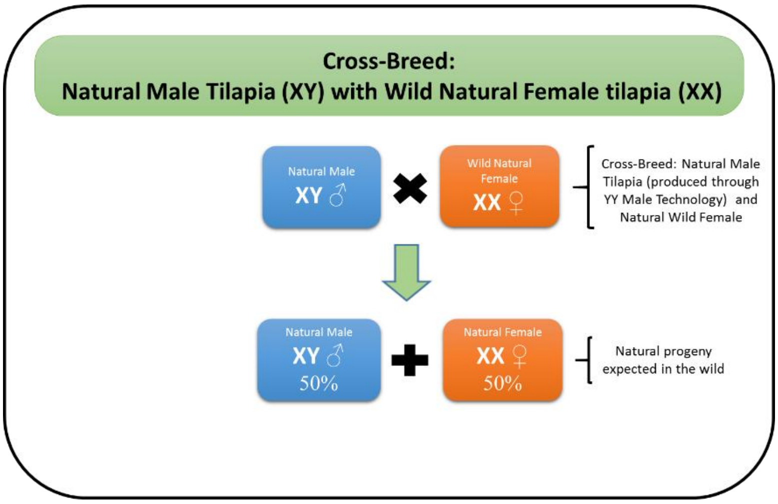

Figure 6 Schematic representation of expected progeny from cross-breeding between Natural Male Tilapias, produced through YY Male Technology, and natural wild females.

YY Male technology has the potential to provide a great array of benefits for the fish farmer, the fisheries, the environment and the fish itself.

- Safer to humans and farm operators, as it avoids the health risks associated with the usage and handling of hormones. Improper hormone handling and accidental contact can be harmful to humans if swallowed, absorbed through skin and/or inhalation, and has a danger of cumulative effects (US Fish and Wildlife Service, 2011). MT is considered to be carcinogenic and can cause liver damage (Velazquez and Alter, 2004). MT is an endocrine disrupter at part per billion levels and can interfere with normal functions of the reproductive systems of humans and animals (Barbosa et al. 2013).
- Safer to the fish, resulting in greater fingerling survival rates as it avoids the health risks affecting the fish associated with hormone usage. Rubio-Godoy et al. (2011) and Conroy (2001) described the negative impact of MT treatment in fingerling survival rates due to higher levels of hecto-parasitic, fungal and bacterial infestations (Gyrodactilus, Trichodina, Saprolegnia, Columnaris, Edwardsiella, etc.) as a result of the fish immune system suppression effect. Additionally, MT administered at high dose can lead to sterility and in some cases paradoxical sex reversal and may result in growth suppression (Pandian et al., 1995).
- Safer to the environment, as avoid any environmental impact risks that hormone residues in the production water, units and system waste could have. These risks have been widely discussed by several scientist (Fitzpatrick et al. 2000, Contreras et al., 2000, Herrera et al., 2001, Macintosh, 2006, Muir et al., 2001. Dunham, 2004, Senanan et al., 2008, Rao et al., 2012, Harris et al., 2000). Hulak et al. (2008) showed that effluents of systems in which carp were fed diets containing MT caused masculinization of female fish.
- Reduced ecological impact of wild populations when farmed stocks are released in the wild through either restocking programs and/or accidental escapees from fish

farming, potentially resulting in subsequential gender ratio imbalances in their offspring when cross-bred with wild populations (Fig 3 vs Fig 6).

- A more consistent and reliable technique to achieve offsprings with high Natural Male ratio (always above 95%) compared to MT treatment (not rare to see male ratios below 90%).
- Promotes offspring heterosis or hybrid vigour as the YY male technique itself requires to keep two different lines between the male (YY) and female (XX) broodstock.
- Increased interest by major markets worldwide (particularly in Europe and recently in the US) for environmentally friendly and sustainable food sources. The application of hormones to fish destined for human consumption is prohibited in the European Union under directive 96/22/EC, article 5, which also prohibits import of animal products produced with hormones (Stadtlander et al., 2013).

Conclusions

The YY male technology provides a robust and reliable solution to the problem of early sexual maturation, unwanted reproduction and overpopulation in tilapia culture, and can increase production of tilapia by 50%, (Beardmore et al., 2001; Dunham et al.,2001, Dunham, 2004).

Mair et al. (1997) described the important comparative advantages and disadvantages of the YY male technology as a means for mass production of monosex male tilapia, as compared to other commonly used alternatives such as manual sexing, hybridisation and sex reversal. The technique can be considered environmentally friendly as no hormones are applied to fish at any stage of the production process. Species\strain purity is maintained and the fish produced for culture are normal genetic males. Although the development process is time consuming and labour intensive, once developed, the production of monosex males can be maintained through occasional feminisation of YY genotypes. Provided that broodstock purity can be maintained, the technology can be applied (at the level of spawning ‘YY’ males with normal females) in existing hatchery systems without any special facilities or labour requirements. Discounting the initial development costs, additional costs for application of this technology at the hatchery level would be minimal, while the potential economic advantages to growers have been demonstrated to be very considerable.

One of the biggest advantages of using the YY-technology is the fact that its masculinization effect ALWAYS works. But another not to neglect advantage is that these all-male population consists of completely normal males (XY). When released in the wild through restocking programs or by accident, the winner is always the local fisherman.

ASC Tilapia Standard (2011) mandate the culture of all male tilapia with a minimum percentage of males per culture unit of 95%. A requirement that only YY male technology can reassure. Both, the ASC tilapia Standard and FAO’s code of conduct for responsible fisheries (1995) make reference to the prevention of adverse genetic effects from escaped farmed fish on wild stocks.

The magnitude of a genetic impact from escapees will depend on the numbers of escapees into the wild compared to their wild counterparts, genetic differences between escapees and wild fish, the outcome of competition for feeding and breeding sites, reproductive success and the fitness of hybrid offspring (Senanan and Bart, 2006). When considering the release of thousands, and some times, millions of SR-tilapia into the wild by governmental restocking programs and/or accidental farm escapees, makes evident the magnitude of its impact into the wild ecosystem. Additionally, by employing Natural Male Tilapia in governmental restocking programs instead of mix-sex populations, the fishermen would be benefited with increased captures, as the 100% male stocks employed will grow faster compared to only 50% of the mix-sex stocks. Also this could help to reduce the fishing pressure into wild populations and promote sustainable fishing practices as fishermen could be enticed to target larger specimens

Mexico accounts for 6,500 km2 of inland freshwater bodies and 714.42 km2 of shrimp farms that could potentially switch into tilapia culture if in trouble. If we consider that at least a 50% of these water bodies could potentially be stocked with tilapia for either fisheries restocking programs or aquaculture, this represent a massive 3.6 billion m^2 of water and therefore a huge potential for tilapia production in Mexico.

Also Ghana, with 8,520 km2 of inland lakes and rivers, has a variety of investment opportunities in the inland fisheries subsector. The fish farming subsector of the national economy will comprises freshwater fisheries and fish farming or aquaculture.

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