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Increasing salinity tolerance in tilapias: selective breeding using locally available strains

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Abstract. The culture of tilapias is an important industry in most aquaculture producing countries in the world. Due to the many favorable attributes of this species of fish, including tolerance to wide range of environmental conditions and fast growth rates, interest in its culture is increasing at a rapid pace. There have been research initiatives on breeding, genetic improvement and stock manipulation in order to increase the resistance of this fish to high salinity levels, enabling them to thrive in brackishwater and almost seawater conditions. Saline-tolerant tilapias have been co-cultured with shrimp in brackishwater ponds as it was believed that tilapias are able to inhibit the occurrence of luminous vibriosis in ponds. This paper describes the selective breeding program that we developed in order to produce high-saline tolerant tilapias by using locally available strains.
Key Words: Tilapia, *Oreochromis* sp., saline-tolerant, breeding.

Introduction. Tilapias are one of the most economically-important groups of aquaculture species because they serve as major sources of protein in most countries. They are versatile species of fish, which is found in almost any type of tropical aquaculture systems ranging from traditional to highly intensive production systems. They withstand wide range of environmental conditions and perform well regardless of the water salinity to which they are exposed to. Some species are even able to thrive and breed in full strength seawater (Cnaani & Hulata 2011).

Among the tilapia species, the Nile tilapia, *Oreochromis niloticus* is the preferred species for culture as this fish dominates production in freshwater ponds and cages (Kamal & Mair 2005). However, it has low tolerance to high salinity levels. On the other hand, the Mozambique tilapia, *Oreochromis mossambicus* is a euryhaline species and is one of the best studied tilapias in terms of elucidating the mechanisms involved in euryhalinity among fishes (Uchida et al 2000). However, Mozambique tilapia exhibits poor growth due to inbreeding of the founder stock. A tilapia hybrid, the Florida red tilapia also grows well in high saline waters and studies have been done in line with some biotechnical and socio-economic aspects of its culture in saline environments (Watanabe et al 2006).

The culture of tilapias in saline waters is well-documented based on numerous research studies done in the past (reviewed in Cnaani & Hulata 2011). Given the limited space for freshwater aquaculture and pressures on providing the food demands of the population, tilapias are now being cultured in brackishwater ponds and even in marine cages. This scenario will further intensify in the years to come in order to cope with food requirements of the increasing human population.

One of the constraints in the culture of tilapias in high saline environments is its sensitivity to handling and susceptibility to secondary infections (Chang & Plumb 1996). Hence, tilapias reared in high salinities experience higher disease outbreaks and mortalities than those that are reared in freshwater environment. Because of these problems, there have been intensive research efforts on improving the salinity tolerance of tilapias either through modifications in the culture techniques or stock improvement.

This paper discusses the breeding program that was developed at our research facility on selective breeding of tilapia using local strains in order to produce saline-tolerant tilapia hybrids.

Increasing Tolerance of Salinity though Selective Breeding. The different approaches on the improvement of tolerance in tilapias to saline environment as well as the underlying mechanisms involved in their salinity tolerance have been discussed by Cnaani & Hulata (2011). The authors stated that salt tolerance of the fish is the overall fitness of that particular species of fish in a saline environment, and is a combination of traits including metabolism, growth, osmoregulation, immunocompetence and fecundity. Each trait is influenced by multiple genes leading to genetic variation. They further stressed that the underlying phenotypic differences in salt tolerance can result in intra- and inter-specific variation among tilapia populations.

The physiological mechanisms in tilapias during exposure to changes in salinity levels have been extensively reviewed (Prunet & Bornancin 1989). *O. mossambicus* is more adaptable to high salinity levels than *O. niloticus* because of the ability of the former to modify the structures of its esophageal epithelial cells during transfer to seawater (Cataldi et al 1988). Other modifications that tilapias undergo when transferred to high salinity levels include functional transformation in the kidney (Cataldi et al 1991), changes in the chloride cell characteristics (Fiess et al 2007), changes in the amino acid composition of the digestive enzymes (Fang & Chiou 1989), changes in the neuroendocrine hormones (Sakamoto & McCormick 2006) and regulation of the different osmosensors (Loretz 2008).

Transcriptomic approaches have also been used to analyze the mechanisms involved in salinity tolerance among tilapias. Fiol et al (2006) analyzed the transcriptional profile of the genes involved in hyperosmotic stress in the gill epithelial cells of *O. mossambicus* during seawater transfer. They found that most of the genes were immediately upregulated following transfer to seawater, and the genes involved in osmotic response can be grouped into six categories, namely, (1) stress response signal transduction, (2) compatible organic osmolyte accumulation, (3) energy metabolism, (4) lipid transport and cell membrane protection, (5) actin-based cytoskeleton dynamics, and (6) protein and mRNA stability.

Selective breeding is one of the approaches that is used to improve the ability of tilapias to withstand high salinity levels. A simple method to evaluate salinity tolerance in parental strains and hybrids of tilapia is imperative when conducting selective breeding programs for salinity tolerance. An example of this method is known as the median lethal salinity (MLS), which is a simple index based on the short-term capacity of the fish to adapt to daily increments of salinity (Lemarié et al 2004). MLS is the salinity at which 50% of the fish population dies at a given daily rate of increasing salinity levels. This method has been successfully used to evaluate salinity tolerance in *O. niloticus* and *Sarotherodon melanotheron*. This technique takes into account the capacity of fish to adapt to increasing salinity levels and is a simple, optimized and efficient criterion for assessing salinity tolerance of tilapias. Selective breeding programs for both purebreds and crossbreds of tilapias in terms of salinity tolerance and growth have been done in the Philippines (Rosario et al 2004; Tayamen et al 2004). Also, quantitative genetics through the use of diallele mating design was employed to evaluate genetics effects influencing salinity tolerance (Armas-Rosales 2006).

Selective Breeding Initiatives Using Local Strains. Tilapias are not endemic to the Philippines but several of the introduced species and strains have become well-adapted to the conditions of the country. We have developed a breeding program for the production of saline-tolerant tilapia hybrids using different strains that are locally available. We produced a saline-tolerant tilapia hybrid by crossing *O. mossambicus* with another tilapia hybrid, *O. spilorus* x *O. niloticus* GIFT x *O. aureu*s. The parental strains, *O. mossambicus* were obtained from the holding ponds of the research center, while the tilapia hybrids were purchased from a commercial tilapia farm in Central Philippines. These two tilapia strains are tolerant to high salinity levels even at full seawater (Jaspe et al 2007); hence, they were good candidates for selective breeding to produce saline-tolerant hybrids. The broodstock were placed in 1 m x 1 m mesh nets, which were installed inside the concrete outdoor tanks, supplied with flow-through freshwater and ample aeration. The fish were fed with a commercial feed twice a day until satiation. During breeding, the male to female ratio is 1:3 in order to facilitate effective fertilization and to ensure that all females will be mouth-brooders. F_1 progenies from the following crosses: (1) *O. mossambicus* (female) crossed with the hybrid, *O. spilorus x O. niloticus* GIFT *x O.aureus* (male), and (2) *O. mossambicus (male)* crossed with *O. spilorus x O. niloticus* GIFT *x O. aureus* (female).

The F_1 progenies of these crosses have been subjected to a salinity test in order to determine their level of tolerance to abrupt changes in salinity and how they cope with the extreme changes. All the F_1 progenies of the parental strains as well as the crosses were able to tolerate abrupt changes in salinity and there were no mortalities in the fish even at a salinity level of 50 ppt over a monitoring period of 20 days (Jaspe et al 2007). During the course of the study, the fish did not develop lesions; thus, there is least likely a possibility of secondary infections. However, we have noticed that when there was an abrupt change in salinity to a maximum increase of 5 ppt per day, the fish were sluggish a few minutes after exposure but recovered a within one hour after and resumed their normal feeding activity. We have also observed that when the salinity level reached at least 30 ppt, the fish had dark pigmentation, showed erratic swimming behavior and stopped feeding but resumed to normal conditions within a few hours after they adjusted to the salinity conditions. In the course of the salinity tolerance test, we maintained the optimum water quality parameters in the containers. One important factor that we considered was water temperature, in which the range was 26-28^{o}C. This optimum water temperature is crucial in maintaining a steady state plasma osmolality in the fish during exposure to salinity changes (Sardella et al 2004) and could also be partly responsible in preventing mortalities in the fish.

Next, we reared to maturity the F_1 progenies of the saline-tolerant tilapia hybrids and assessed their reproductive potential. An assessment of the reproductive capacity of the fish is needed in order to find out if the resulting hybrids are able to support the fry requirement during the grow-out phase. Also, this information is necessary to ensure that there is sufficient number of broodstock to be maintained in the hatcheries. Mature eggs were collected from the female spawners of the F_1 of the parental strains (*O. mossambicus* and the tilapia hybrid, *Oreochromis spilorus* x *O. niloticus* GIFT x *O. aureus*) and their crosses. We observed that among the reproductive parameters that were assessed, total fecundity and gonadosomatic varied among the spawners but not the relative fecundity. Total fecundity is positively correlated with the body weight of the spawner; thus, when choosing the spawner for selective breeding, it is important to take into consideration the size of the fish. Since we did not find any significant difference in the relative fecundity, that is, the number of eggs produced per unit weight of the fish, therefore any combination of the parental strains during selective breeding can be used to produce saline-tolerant progenies.

In terms of the performance of the F1 progenies in the grow-out ponds, our initial observations showed that the growth, survival and production of the tilapia hybrids did not vary significantly, therefore combinations of the parental strains during selective breeding do not affect the grow-out performance of the resulting progenies. We are currently mass producing these saline-tolerant strains in order to establish an effective number of the founder stock to prevent depression in growth of future generations due to inbreeding. A flow-chart for this breeding program is shown in Figure 1.

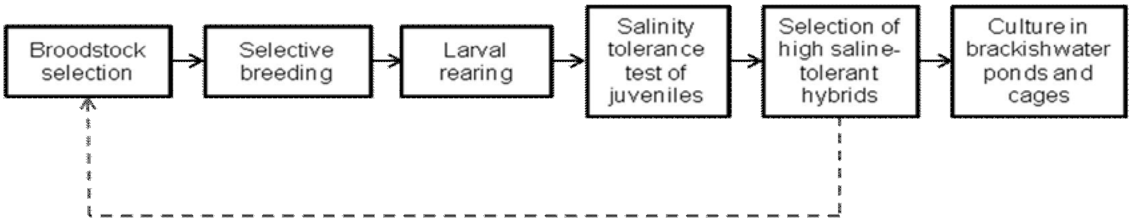

Figure 1. Schematic diagram of the breeding program for locally-available strains of tilapia.

Aside from the establishment of the selective breeding program for the production of saline-tolerant tilapia strains, the water obtained from the broodstock tanks of the saline-tolerant tilapia strains is a good source of "green water". It is called "green water" because it contains considerable populations of green microalgae such as *Chlorella* sp. (Tendencia et al 2005). In a small-scale study, the "green water" that we obtained from the broodstock tanks of the saline-tolerant tilapias was able to inhibit the proliferation of *Vibrio harveyi*, the causative agent of luminous vibriosis in shrimp aquaculture (Huervana et al 2006). As such, the saline-tolerant tilapia strains that we produced in our selective breeding program can be cultured together with marine shrimp in ponds for the production of the "green water technology", thus, preventing future outbreaks of luminous Vibriosis (Corre et al 2000; Cruz et al 2008).

Conclusions. We have developed a selective breeding program for the production of saline-tolerant strains of tilapia using parental strains that are available locally. The salinity tolerance, growth performance and survival of the F_1 progenies from the different crosses were not significantly different; hence, any combination of the parental strains during spawning can be used without affecting the physiological characteristics of the offspring. In addition, the saline-tolerant hybrids that we produced can be a good source for the green water technology in shrimp aquaculture and is a good management strategy to control bacterial diseases such as luminous vibriosis during the shrimp grow-out phase.

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