ABAH BIOFLUX

Animal Biology & Animal Husbandry
International Journal of the Bioflux Society

Small-scale hatchery and larval rearing techniques for local strains of saline-tolerant tilapia, *Oreochromis* spp.

[1]Cecilia J. Jaspe, and [2]Christopher M. A. Caipang

[1] Brackishwater Aquaculture Center, Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miag-ao 5023, Iloilo, Philippines; [2] Faculty of Biosciences and Aquaculture, University of Nordland, Bodø 8049, Norway. Corresponding author: C. M. A. Caipang, cmacaipang@yahoo.com

Abstract. Tilapia aquaculture is an important industry in most tropical and subtropical countries in the world. This species of fish is able to tolerate a wide range of of environmental conditions and has fast growth rates, making it a suitable candidate for aquaculture in developing countries. Tilapias are now being cultured in brackishwater ponds, where salinity levels could reach seawater strength during the summer months. Hence, research efforts have been directed towards the production of saline-tolerant strains. Using locally available strains of tilapias, we developed a small-scale hatchery for the production of saline-tolerant tilapia fingerlings. This paper describes the protocol of this small-scale hatchery production system and larval rearing techniques for the saline-tolerant tilapias. It outlines the detailed procedures from broodstock selection and maintenance, hatchery management and larval rearing including production of all-male offsprings through dietary inclusion of 17-α methyl testosterone and acclimation to high salinity levels.

Key Words: tilapia, *Oreochromis* sp., hatchery, larval rearing, saline-tolerant.

Introduction. Tilapias are considered one of the most important aquaculture species and represent a major source of protein in many countries, especially in the tropical and subtropical regions. The production of tilapia has increased rapidly over the past decade, and different species of this fish have been stocked and transplanted in many countries (Eknath et al 1993).

The culture of tilapias in saline waters is well-documented based on numerous research studies done in the past (Cnaani & Hulata 2011). With limited space for freshwater aquaculture and increasing pressures to provide 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.

Among the tilapia species that are widely cultured, the Nile tilapia, *Oreochromis niloticus* 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 but 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).

One of the constraints in the culture of tilapias in high saline environments is its sensitivity to handling and 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.

Another constraint in tilapia cultura is the availability of year-round supply of seedstock, which hinders expansion of tilapia production (El-Sayed & Kawanna 2007). Tilapias have low fecundity and are asynchronous spawners (Ridha & Cruz 2000). These negative attributes of the fish result in the shortage of seedstock to meet the requirements of the culture industry. As such, the hatchery system provides the backbone and is an important component in the aquaculture production (Ridha 2004). The establishment of small-scale or backyard tilapia hatcheries aims to ensure that there is a steady supply all year round of high quality tilapia fry for the fish farmers in a particular locality.

This paper discusses the development of a small-scale hatchery system for the production of saline-tolerant strains of tilapia. The protocol is briefly described from the selection of broodstock, breeding and hatchery techniques and larval rearing that was developed at our research facility in the Philippines. Through this initiative, we are able to ensure continuous supply of tilapia fry to the fishfarmers as well as to indirectly encourage the culture of tilapias in brackishwater ponds and cages because fish farmers are assured of the availability of saline-tolerant tilapia fry.

Reproductive Physiology of *Oreochromis* spp. Tilapiine fishes are categorized according to their mode of reproduction (Coward & Bromage 2000). The most recent reclassification of tilapia taxonomy was made by Trewavas (1983), which adopted three generic groupings, namely, *Tilapia* (substrate spawner/guarder), *Sarotherodon* (biparental mouthbrooders) and the *Oreochromis* (maternal mouthbrooders). This reclassification is based principally on their mode of reproduction but also differences in feeding habits and biogeographical distribution were taken into consideration.

Majority of tilapias that are utilized for aquaculture belong to the genus *Oreochromis* (Coward & Bromage 2000). Communal breeding takes place, and the males usually build and defend territories within a defined spawning area. There is a rather short courtship between the resident males and the visiting females, and this could last for a few hours (Rana 1988). This results in the deposition of the eggs in the shllow nests and are eventually picked up by the female and incubated in her buccal cavity. The female then leaves the nest and takes care of the egg clutch until the fry reach the free-swimming stage (Rana 1988). At the free-swimming stage, the females exhibit extended periods of maternal care, in which the free-swimming fry can seek shelter in the mouth of the female when they sense danger (Coward & Bromage 2000).

The reproductive physiology of the maternal mouthbrooding tilapia, *Oreochromis* spp. poses some negative characterisitics including low egg production, asynchronous spawning and limited production of seedstock (Rana 1988; Little et al 1993). These characteristics are limiting factors in the expansion of the tilapia culture industry. To meet the seed requirement of the industry, a large number of broodstock has to be maintained (Ridha 2004), but this can considerably increase production costs for maintenance. There have been a number of techniques that can alleviate the problems associated with low fecundity and asynchronous spawning. These include manipulation of environmental conditions such as temperature and photoperiod (Ridha et al 1998; Campos-Mendoza et al 2004; El-Sayed & Kawanna 2007), age and size at first maturity (Duponchelle et al 1998; Getinet 2008), density and sex ratio (Little & Hulata 2000), artificial incubation of eggs (Siraj et al 1983), frequency of seed removal (Ridha & Cruz 1998) and dietary manipulations (Gunasekera 1996; El-Sayed et al 2005). Egg production in tilapias could also be influenced by the overall spawning capacity of the group (Desprez et al 2008). This spawning capacity is dependent on the females that spawn more than the others (Mires 1982), which likely suggests the influence of social and behavioral interactions in a group of breeders (Baroiller & Jalabert 1989).

Tilapias also vary in their reproductive potential and seed production rate (Little 1989) and these differences are evident among different strains of the same species (Eguia 1996). Hence, hatchery operators are likely to utilize tilapia strains that are shown to have high seed production rates.

Hatchery Production for Saline-Tolerant Strains. 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 through selective breeding. This was achieved 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. We have sufficient number of these tilapia broodstock in order to maintain the effective number, thus preventing the negative effects of inbreeding. 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.

Rectangular concrete tanks situated outdoors and directly exposed to sunlight are considered to be suitable in the production of sex-reversed saline-tolerant tilapia. These tanks are supported with bamboo roofings and covered with net to prevent debris from getting inside the tanks and to prevent the breeders from jumping out of the tanks. The tanks are used for the following purposes: (1) conditioning of the broodstock (conditioning tank with a dimensión of 5m x 5m), (2) breeding and spawning (spawning/breeding tank with a dimensión of 6m x 7m), and (3) larval rearing and hormone treatment of the fry (larval tank, with a dimensión of 5m x 5m).

Prior to the hatchery operation, all the tanks are cleaned to remove dirt and debris from the previous production run. The tanks are disinfected with chlorine (50 ppm), subsequently rinsed with freshwater and sun-dried for a few days. When the tanks are completely dried, freshwater from a nearby well or groundwater is readily pumped into the individual tanks. Aeration is provided in each tank to condition the water prior to stocking the broodstock. All tanks are subjected to natural ambient rhythm of photoperiod and light intensity.

Brackishwater, which is obtained from a nearby brackishwater river is also available. It is pumped directly to a reservoir, then allowed to pass through a sand filter before being stored in brackishwater supply tanks that are located indoors. During acclimation to increasing salinity levels, brackishwater is evenly distributed to the larval rearing tanks and mixed with freshwater to attain a desired salinity level.

We maintain tilapia broodstock in the brackishwater ponds at our research facility. The fish subsist on both natural food composed mostly of benthic organisms and filamentous algae, and commercial pelleted feed at a crude protein content of at least 10%. At about 6-8 months after stocking in ponds, the breeders are collected and those weighing at least 70 g are selected and transported to the hatchery for conditioning. Based on our experience, tilapias that are of this size are ideal candidates for good egg production. The number of breeders to be collected will depend on the number of tilapia fry needed in each production run. Usually two sets of breeders are collected and bred on rotation for an annual production cycle, then restocked and re-conditioned in the same holding ponds.

Individual breeders are manually sexed and the male and female breeders are segregated and stocked in separate conditioning tanks for a period of two weeks. This is the conditioning phase of the production cycle where *ad libitum* feeding is implemented. High crude protein (at least 30%) feed is advantageous to obtain high egg production and good quality fry.

During the conditioning phase, fine mesh (hapa) net enclosures with a dimension of 2.0m x 1.5m are installed in the breeding/spawning tanks. The nets are tied in four corners of the tank and the bottom corners are attached with sinkers. The water in the breeding/spawning tank is filled up to three-fourths of the total tank capacity.

After 15 days of conditioning, the male and female breeders are transfereed to individual mesh nets inside the breeding/spawning tank. Each mesh net is stocked with breeders at a male:female ratio of 1:3. Usually, a 2m x 1.5 mesh net can be stocked with at most 28 breeders. The breeders are fed with a high protein (crude protein of at least 30%) diet at approximately 25% of the body weight. The feed ration is adjusted to 5% when mouth brooding begins, which usually occurs on the 10^{th} day after stocking in the mesh nets.

Representative samples of the mouthbrooding females are examined on the 10th day to check the stage of the eggs or of the yolk sac fry. This is to determine the expected date that the fry will be released by the brooders as well as the time of collecting the undifferentiated tilapia fry. Usually during the summer months, females produce eggs quite early, usually in about 15 days after stocking in nets inside the breeding/spawning tanks as long as there is sufficient supply of freshwater. However, during the cold/rainy months, breeding could extend up to 1 month.

Collection of the swim-up fry, which tend to gather along the corners of the fine mesh nets, is done using a small scoop net and placed in basins with aeration. This usually happens on the 10th day after stocking in the mesh nets inside the breeding/spawning tanks. At this stage, the breeders are also removed from the mesh nets, their mouths are opened and the operculum is flushed with water to release the fry in the basin. The swim-up fry should not be held in the basin for a long period of time. They must be immediately stocked in larval rearing tanks containing freshwater. On the other hand, the yolk sac fry that were taken from the mouth of the female breeders, are kept in basins with aeration for a number of hours until they reach the swim-up fry stage. During this time, they are transferred to the larval rearing tanks. Since not all females will spawn at the same time, brooders with unhatched eggs will be restocked in the same mesh nets until hatching of the eggs.

Figure 1 shows the layout of a small-scale hatchery for the production of saline-tolerant tilapia strains. This is the set-up at our research facility, in which there are two conditioning tanks, and spawning/breeding tanks as well as larval rearing tanks. The ratio of 1:3 for the number of larval rearing tanks to the breeding/spawning tanks is an ideal set-up to produce at least 50,000 tilapia fry during one hatchery production run.

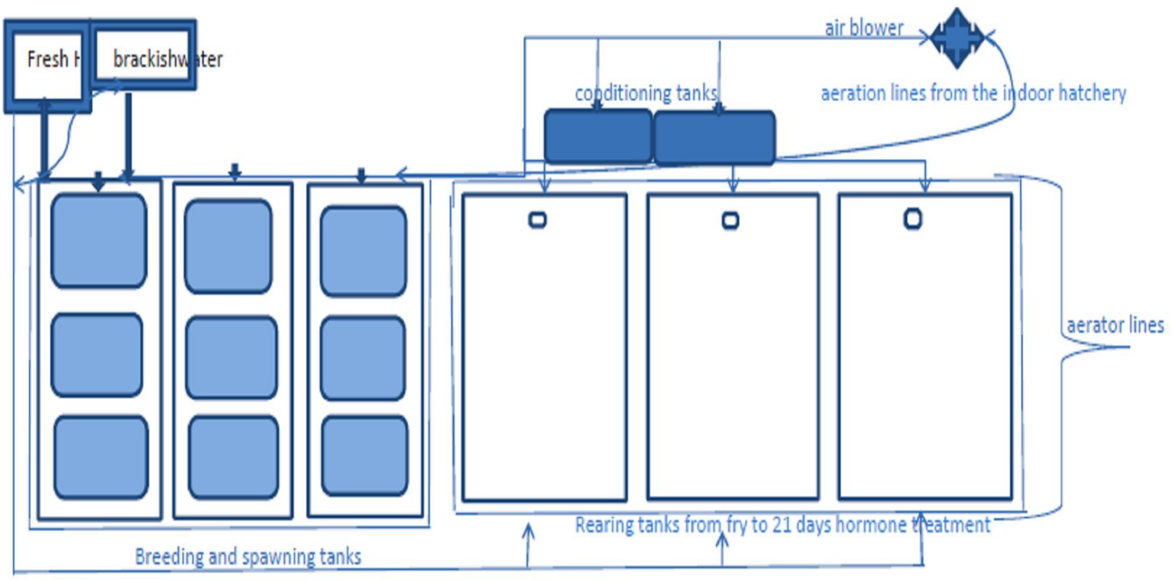

Figure 1. Lay-out of a small-scale hatchery for the production of saline-tolerant tilapia strains.

Larval Rearing in Tanks or Net Enclosures. Yolk-sac fry that are collected from the net enclosures in side the breeding/spawning tanks are transferred to the larval rearing tanks. The tanks are stocked with the same batch or age of the yolk-sac fry to ensure uniformity of hormone treatment when feeding. During this period, undifferentiated tilapia fry are fed with a commercial feed that is treated with 17-α methyl testosterone (60 mg per Kg of feed) for 21 days. The purpose of incorporating methyl testosterone in the diet of the undifferentiated tilapia fry is to produce an all-male tilapia population, which exhibit faster growth rates compared with females. Because tilapias are precocious spawners (Coward & Bromage 2000), having an all-male population during the culture period will prevent the production of tilapia fry in ponds, thereby reducing the risk of competition for food and space.

The preparation of the feed includes: weighing of 1 kg of powdered or finely ground commercial tilapia feed (crude protein of at least 25%) followed by the addition of 60 mg of 17-a methyl testosterone disolved in 200 ml of food grade denatured alcohol. The powdered feed and the hormone solution are mixed thoroughly and air-dried until the alcohol has completely dissipated. Once traces of alcohol are completely removed, the feed is placed in clean plastic bags, sealed, placed at 4^oC and protected from light. If the feeds are to be used during the same day, the processed feeds should be air-dried for at least 6 hours before feeding to the tilapia fry.

The medicated feed is given every hour or 8 times a day and must be strictly observed. The fry are fed until satiation. Acclimation to brackishwater or saline water is carried out inside the larval rearing tanks. From a starting salinity of 0 ppt, the salinity levels are gradually increased for 3-5 ppt per day until it reaches a final salinity of 30-35 ppt. This acclimation process was a result of our trials done on the different strains of tilapia that we have used in our research facility (Jaspe et al 2007). One important factor that we considered was water temperature, in which the range was $26\text{-}28^oC$. 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.

Harvest is done after 21 days of hormone feeding. At this time, about 95% of the fingerlings are already sexually transformed to an all-male population of saline-tolerant tilapia strains.

The annual production of the saline-tolerant tilapia fry from our small-scale hatchery is shown in Table 1. Using at least 400 tilapia breeders at a male:female ratio of 1:3, we were able to have four hatchery production runs in a year producing at an annual fry production of at least 250,000 fry. The low fry production during Cycle 1 and 2 took place during the cold/rainy months, while Cycle 1 and 4 were carried out during the summer months, hence high fry production was observed.

Table 1

Annual production of saline-tolerant tilapia fry in a small-scale hatchery

Production cycle	*Number of females*	*Number of males*	*Total number of tilapia fry produced*
1	387	121	79,000
2	305	101	57,700
3	246	82	43,050
4	363	121	76,320

We compared the performance in grow-out ponds of the F1 progenies from one hatchery production run. 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.

Water Management. Water exchange in all the tanks is done thrice a week at 50% of the level in the tank. Important water quality parameters such as disolved oxygen, temperature and pH are monitored daily in the morning. Ammonia-N levels are determined weekly. All the water quality parameters are always kept at the optimum levels to ensure high survival and fast growth of the fish.

Conclusion. In summary, we have established a small-scale hatchery system for the production of saline-tolerant tilapias using locally available strains. A similar kind of this system is recommended to be adopted in other tilapia-producing areas in order to sufficiently augment the fry requirements during the grow-out phase.

Acknowledgements. The authors would like to thank the research staff at the Brackishwater Aquaculture Center of the Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas for the technical support, and to the Faculty of Biosciences and Aquaculture, University of Nordland for providing logistical support during the preparation of the manuscript.

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Received: 06 May 2011. Accepted: 01 June 2011. Published online: 04 June 2011.
Authors:
Cecilia J. Jaspe, Brackishwater Aquaculture Center, Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miag-ao 5023, Iloilo, Philippines, salus1_ph@yahoo.com
Christopher Marlowe A. Caipang, Faculty of Biosciences and Aquaculture, University of Nordland, Bodø 8049, Norway, cmacaipang@yahoo.com
How to cite this article:
Jaspe C. J., Caipang C. M. A., 2011 Small-scale hatchery and larval rearing techniques for local strains of saline tolerant tilapia, *Oreochromis* spp. ABAH Bioflux **3**(1):71-77.