Aquaculture 344-349 (2012) 181–187

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Effects of triploidy induction on growth and masculinization of red tilapia [*Oreochromis mossambicus* (Peters, 1852) × *Oreochromis niloticus* (Linnaeus, 1758)]

Padmaja Jayaprasad Pradeep [a,b,*], Thekkeparambil Chandrabose Srijaya [a], Alessio Papini [c], Anil Kumar Chatterji [a]

[a] Institute of Tropical Aquaculture, University Malaysia Terengganu, 21030, Kuala Terengganu, Malaysia
[b] Oral Cancer Research & Coordinating Centre (OCRCC), Faculty of Dentistry, University of Malaya, 50603 Kuala Lumpur, Malaysia
[c] Department of Evolutionary Biology, University of Florence, Via La Pira, 4 — 50121 Firenze, Italy

ARTICLE INFO

Article history:
Received 2 September 2011
Received in revised form 1 March 2012
Accepted 7 March 2012
Available online 15 March 2012

Keywords:
Masculinization
Red tilapia
Sex ratio
Sterility
Triploidy

ABSTRACT

The present study aims to evaluate the effects of triploidy induction by temperature-shocks (heat shock at 41 °C for 5 min and cold shock at 9 °C for 30 min of duration, starting 4 min after fertilization) on growth parameters and the sex ratios in progenies of three different red tilapia brood stocks under tank culture condition for 120 days. A significant difference in total yield ($P<0.05$) was recorded in favor of the heat-shocked induced triploid groups. Total average body weight of male fish in all the groups showed more weight gain than the females, during the culture period ($P<0.01$). Statistical analysis of the total weight gain among the sexes revealed that there was no significant difference between the average weights of male belonging to various groups, whereas in females the heat-shocked group showed a significant increase with respect to that of diploid females ($P<0.05$). Red tilapia subjected to heat-shock treatment showed positive correlation ($P<0.001$) with sex ratio, where skewness towards male progenies (84.1%) was observed as compared to cold-shocked and control groups. Highly significant difference in ovary weight and GSI was assessed between triploidy induced females and diploid females of the control group ($P<0.001$). The impact of heat shock and cold shock induction of triploidy on the performance and masculinization is discussed in detail.

1. Introduction

The curiosity behind the sex determination mechanism in tilapia is motivated by the practical and commercial implications in the production of monosex male populations for aquaculture (Desprez et al., 2006). The sex differentiation into male or female is a complex and labile mechanism under genetic control including a mixture of major (xx/xy and zz/wz) and minor factors (autosomal) (Baroiller et al., 1999, 2009; Devlin and Nagahama, 2002; Lee et al., 2003; Rougeot et al., 2008). Many other external factors such as high and low water temperatures, low pH, salinity; several physiological factors like nutrition, crowding and social cues and exogenous steroid hormones have also been shown to modify the phenotypic sex of an individual and consequently, alter the sex ratios of the offspring (Abucay et al., 1999; Baroiller and D'Cotta, 2001; Baroiller et al., 1996a, 1996b; Baroiller et al., 1999; Desprez et al., 2003; Nagahama et al., 2004; Rubin, 1985; Sullivan and Schultz, 1986; Tessema et al., 2006). Most of the work on environmental sex determination in tilapia has focused on the effects of temperature. Earlier studies on various tilapia species have shown that high-temperature treatments during the early developmental stage (labile period) cause a significant skewness of sex ratio in favor of males (Baras et al., 2000, 2001; Baroiller et al., 1995, 1996a, 1996b; Desprez and Me' lard, 1998) while lower temperature treatments resulted in skewness towards female (Baroiller et al., 1996b). These findings suggest that an early sex differentiation pathway during the embryonic stage is prevailing in tilapia (Rougeot et al., 2008). However, there is a paucity of information regarding the various environmental effects on sex differentiation during the embryonic development before hatching (Rosenstein and Hulata, 1992).

Production of sterile tilapias through triploidy induction has attracted considerable interest in the past (Mair, 1993). All the earlier studies have reported that the high levels of triploidy can be achieved in tilapia using various shock treatments soon after the fertilization (Chang and Liao, 1996; Chourrout and Itskovich, 1983; Don and Avtalion, 1986, 1988; Hussain et al., 1991; Penman et al., 1987; Valenti, 1975; Varadaraj and Pandian, 1988). Production of all-female sterile triploid tilapia has been attempted by Varadaraj and Pandian (1990) and they have suggested that all female-sterile tilapia may have considerable potential for tilapia aquaculture. In fact, production of sterile triploid males can also be considered as a holistic approach to an alternative method for monosex technique. All-male tilapia populations are a desirable solution to control the prolific

* Corresponding author at: Institute of Tropical Aquaculture, University Malaysia Terengganu, 21030, Kuala Terengganu, Malaysia. Tel.: +60 129318636; fax: +60 3 79561607.
E-mail address: pradeep85pj@yahoo.com (P.J. Pradeep).

0044-8486/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2012.03.006

breeding activity. Moreover this solution improves the yield in tilapia aquaculture, since the male grows faster than the female in this species (Macintosh and Little, 1995; Myers et al., 1995; Tessema et al., 2006). Furthermore, application of monosex in tilapia aquaculture would offer several other advantages like; reduction of sexual/territorial behavior, reduction of variation in harvest size, and reduction of risk of environmental impact resulting from escape of exotic species (Beardmore et al., 2001).

Previous studies on triploidy induction in tilapia, has shown to select female sex (Byamungu et al., 2001; Mol et al., 1994). However, no reports have ever been made on the influence of temperature shocks during triploidy induction in altering the sex ratios of tilapia in favor of males. Since temperature shocks are applied soon after the fertilization, possibility of modifying the sex ratios right from the time of fertilization may be possible. Hence, comprehensive experiments were carried out with the aim to evaluate the effects of temperature shock triploidy induction on sex ratios and growth in red tilapia.

2. Materials and methods

2.1. Origin of fish and brood stock management

The experimental fish used in this study were commercial hybrid red tilapia strain commonly known as red tilapia, *Oreochromis mossambicus* (Peters, 1852) × *Oreochromis niloticus* (Linnaeus, 1758). Fresh stocks of red tilapia adult males and females used in the present study were obtained from a cage culture farm located in the Lake Kenyir, Terengganu in Malaysia. This study was carried out at the Freshwater Aquaculture Unit of University of Malaysia in Terengganu. Brooders were fed twice a day with pelleted feed (43% protein, ASEAN Marine Fish Feed, Ltd). In addition to ASEAN Marine Feed, fish were also given *ad-libitum* with *Cabomba caroliniana* once in a week to satisfy their vegetative requirements. The fish were maintained under natural conditions of photoperiod and temperature (26–27 °C) in 5000 l rectangular cement tanks with proper aeration. A defined water quality were kept in the tank with oxygen > 7 mg/l; pH 6.5–7.0; NH_4^+ < 0.25 mg/l; and NO_2^- < 0.2 mg/l.

2.2. Spawning

Before starting the experiments, the brooders were starved for 24 h. Three pairs of brooders [males (average weight: 223.7 g) and females (average weight: 236.4 g)] were selected from the cement tanks on the basis of readiness for spawning as suggested by Rothbard and Pruginin (1975). For better ovulation and spermiation, the selected brooders were induced by HCG (Pregnyl1500) injection (1500 IAU/kg body weight) which was applied just below the dorsal fin. Each pair of fish was kept in 120 l-aquarium tank. The fish were kept separated by a sheet of Perspex. The aquarium tank was provided with constant water temperature (28 ± 1 °C) controlled by a digital heater (Model-D-38300, 300 W-Italy) and adequate aeration. Ovulation and spawning readiness during the experiment time were determined by observation of courtship behavior, coloration and papilla erection (Rothbard and Pruginin, 1975).

2.3. Gamete collection

Females were stripped after the initiation of spawning, *i.e.* after the release of the first batch of eggs (~20–30 nos.). Simultaneously, the partner male was also stripped. Soon after the gamete collection, the eggs were fertilized with 0.6–1 ml of milt diluted with a small quantity of freshwater (28 ± 1 °C). After a minute, the eggs were rinsed with fresh water to avoid polyspermy and then divided into control and experimental batches.

2.4. Triploidy induction

From each brood stock pair (N = 3), *in vitro* fertilized eggs were collected and divided into three equal batches consisting of 350–400 eggs/batch. The shock treatment on fertilized eggs collected from each pair was considered as replicates. Two batches were used to produce triploids (3N) by heat (41 °C for 5 min duration, 4 min after fertilization) Pradeep et al. (2010) and cold (9 °C for 30 min duration, 4 min after fertilization) shocks (Pradeep, 2011). The third batch was untreated and was considered as a normal diploid control (2N).

2.5. Egg incubation and calculation of survival

After the shock induction, eggs were counted and transferred to the incubation chamber along with control group for further development. All fertilized eggs of both treated and control groups were incubated identically in round bottomed glass jars (250 ml) connected to a re-circulatory incubation system (Pradeep et al., 2011a). The fertilized eggs were counted at the blastula stage *i.e.* 10 h after the fertilization (a.f.) and hatching rate at 80–90 h a.f. The survival rates were taken on the 5th day (120 h a.f.) and 120th day, during the termination time of the experiment.

2.6. Ploidy evaluation

Ploidy evaluation was performed by chromosome preparations from one day old red tilapia larvae. Chromosome counts were made on a subsample (N = 10) from each batch (Pradeep et al., 2011b). Further verification of the ploidy was done when the fish had reached 60 days of age using mean cellular volume of the erythrocytes (Pradeep et al., 2011c). The blood samples of each fish from all treatments were collected and verified for ploidy, without killing the fish.

2.7. Fry and fingerling rearing

After hatching, 220 larvae (0.011 g/larva) were removed from each group and stocked separately in nine glass aquarium tanks (550 l). The temperatures of the aquarium tanks were maintained at 28 ± 1 °C using digital heater (Model-D-38300, 300 W-Italy) until they were transferred to cement tanks (age 30 days) for further development. Initially, larvae were fed with newly hatched *Artemia nauplii* at a rate of 10–15 individuals/ml, 3–5 times a day for a week. This was followed by feeding the fries with powdered feed (ASEAN Marine Fish Feed with 43% protein) at a ratio of 12–15% of their body weight, thrice a day for a period of 10 days. After the 30th day, fries (N = 180) were transferred to cement tanks (3.5 × 2 × 2 m) where the level of water was constantly maintained at 1.5 m throughout the experimental period. The feeding rate was subsequently reduced to 8–10% of the total biomass allowing fishes to feed 2–4 times a day. The feeding program was re-scheduled after the 40th day and there after the fish was fed twice with commercially available sea bass pelleted feed at 5% of their body weight. The daily ration was calculated every 15 days and for that purpose the average body weight of 20 fish in each tank was taken and accordingly rationing was determined using the following equation:

$$\text{Daily food ration (DFR)} = \text{Average body weight} \times \text{No.of Individuals} \times \% \text{ food requirement}.$$

2.8. Water quality management

All the nine batches (3 treatments with their replicates) were maintained under similar culture condition in the cement tanks to avoid any experimental error. Water quality was maintained throughout the