Aquaculture Reports 3 (2016) 45–50

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

An optimized group mating design and determination of the admixture rate in Nile tilapia families

Yan Zhao[a,1], Hong-ju Chen[a,1], Chun-Ge Li[a], Hui Wang[a], Hong-Jun Liu[b], Xiang-Shan Ji[a,*]

[a] College of Animal Science and Technology, Shandong Agricultural University, Taian 271018, China
[b] Marine Biology Research Institute, Shandong, China

ARTICLE INFO

Article history:
Received 24 May 2015
Received in revised form
30 September 2015
Accepted 26 November 2015
Available online 9 December 2015

Keywords:
Nile tilapia
Family
Microsatellite
Parentage analysis

ABSTRACT

The Nile tilapia is the most productive and internationally traded food fish in the world. Many Nile tilapia families are needed to develop breeding programs for this species. The aim of this study was to develop Nile tilapia families using the *Oreochromis* species unique breeding characteristics (male territory establisher and female mouth brooders) and determine the mixing rate within each family. Both male and female fish were cultured in one pond in the reproductive season and the female mouths were checked every 5–8 days. When embryos were present, they were removed and cultivated independently as a family. As a result, 45 families were developed from the 60 female fish sampled. The embryo survival rate in aerated water varied from 85.5 to 100.0%. Seven polymorphic microsatellite loci were selected from a set of 31 for paternity identification. The CPE (combined paternity exclusion probability) of two microsatellite loci was higher than 0.93 and the CPE for the seven loci used in this study was as high as 0.9999. There were no unrelated individuals in five of the seven families analyzed and only one mixed individual in F29 and F30. The overall admixture rate was very low (2.2%). In this study, we developed an optimized group mating system. Furthermore, we verified that for group mating systems there were no mixed individuals in most families and the admixture rate of some families was very low according to the paternity analysis.

1. Introduction

The Nile tilapia, *Oreochromis niloticus*, is the most productive and internationally traded food fish in the world. The Nile tilapia breeding program to improve growth has been ongoing for almost 30 years (Eknath and Acosta, 1998). A 10-year multi-national effort for genetic improvement led to the development of the highly successful GIFT (Genetically Improved Farmed Tilapia) strain (Ponzoni et al., 2005). The estimated total genetic change of GIFT in live weight was 64% over nine generations (Khaw et al., 2008). Additionally, several other strains of Nile tilapia have also been developed globally, e.g., the new GIFT strain in China (Tang et al., 2013). Currently, scientists all over the world continue to select Nile tilapia to improve growth (Khaw et al., 2012; Nguyen et al., 2010). Because within-family selection is often used, high numbers of Nile tilapia families are required.

The Nile tilapia families are used in other respects: reliable estimates of genetic parameters (e.g., heritability and genetic correlation) for important breeding target traits (Tian et al., 2011); the development of genetic and physical Nile tilapia maps to support the isolation of genes controlling economically important traits (Lee et al., 2005); and the production of strains with high percentages of male Nile tilapia with high-temperature treatment (Wessels and Horstgen-Schwark, 2007; Baroiller et al., 1995; Tessema et al., 2006). However, developing families by artificial fertilization is a time- and labor-consuming procedure. The species *Oreochromis* has an elaborate breeding behavior. In most cases, male fish establish and aggressively defend territories (Coward and Bromage, 2000). Nests are built in the form of shallow pits on pond bottoms, and are used for courting and spawning. After the female fish releases her eggs and fertilization takes place, female tilapia will pick up the eggs from the nest, incubate, and protect their young in their mouths (mouth brooders). These unique breeding characteristics make the design of a simple and highly efficient method for developing Nile tilapia families possible. Nile tilapia families have been produced by stocking one male fish with two female fish in small

* Corresponding author. Present address: 61, Daizong Street, College of Animal Science and Technology, Shandong Agricultural University, Taian 271018, Shandong, China. Fax: +86 4102348854.
E-mail address: xsji@sdau.edu.cn (X.-S. Ji).
[1] Contributed equally.

http://dx.doi.org/10.1016/j.aqrep.2015.11.007

hapas (ICLARM, 1998). However, developing families by stocking a number of male and female fish together in one pond has been unsuccessful to date.

The objective of this study was to develop Nile tilapia families by stocking 20 female fish and 6–10 male fish in one pond, assess the admixture rate, and determine the true mating conditions in their natural habitat.

2. Materials and methods

2.1. Experimental fish

Eighty-four 2-year-old Nile tilapia, 60 female and 24 male fish, were obtained from broodstock kept in the Shandong Freshwater Fisheries Institute (Jinan, China). The experimental fish were divided into three groups (20 female fish and 6–10 male fish in each group) and cultured in three ponds (6 m long × 3 m wide × 1 m deep). The ponds were located outdoors and the water temperature ranged from 22 to 30 °C. The Nile tilapia were fed standard carp pellets twice daily. Half of the pond water was exchanged once weekly.

2.2. Fertilized egg collection and hatching

The female mouths were checked every 5–8 days. If there were embryos in the mouth, they were removed and counted. The fin clips were then collected and labeled from the female parent for DNA extraction. The developmental stage of the embryos was determined under a dissecting microscope according to the following criteria: gastrula stage was characterized by the presence of the germ ring around the margin of the blastoderm and the embryonic shield; segmentation stage was characterized by somite formation and the partitioned brain; the pharyngula stage was characterized by the primordia of the pharyngeal arches (Fujimura and Okada, 2007). The embryos of each family were then placed in a hapa (30 cm long × 20 cm wide × 30 cm deep) and incubated in a 0.5-m^3 container at 28–29 °C with an aerating stone. When the embryos had developed to the swimming stage, the fry were counted to determine the percentage of surviving individuals. At the end of the reproductive season, fin clips were collected from all of the male Nile tilapia and stored for DNA extraction.

2.3. Extraction of genomic DNA, primers, and PCR

Genomic DNA from the female parents, all of the male fish in pond two, and 10–20 individuals from each family was extracted from the fins using the phenol/chloroform extraction method modified from Ma and Chen, (2009). Genomic DNA was dissolved in ddH_2O to a final concentration of 100 ng μL^{-1}.

Twenty-six microsatellite loci were screened from published microsatellite sequences in the Tilapia genetic linkage map (Lee et al., 2005). Another five microsatellite sequences were obtained by blasting the Nile tilapia genome databases against sex differentiation-related gene sequences submitted to the NCBI (Table 1). The five sex differentiation-related genes were Cyp19a1a (Cytochrome P450, family 19; ovarian aromatase), Cyp19a1b (Cytochrome P450, family 19; brain aromatase), Amh (anti-Mullerian hormone), Esr1 (estrogen receptor 1), and Sox9a (SRY-box containing gene 9a). The microsatellite loci located in the open reading frame region of Esr1 and Sox9a, intron of Amh, 5′-untranslated regions of Cyp19a1b, and the 3′-untranslated region region close to Cyp19a1a were selected. All of the 31 pairs of primers were designed using Primer Premier 5.0 and synthesized by Shanghai Sangon Co., Ltd. (Shanghai, China). Additionally, the forward primer for the microsatellite locus Amh was labeled with 6-FAM fluorescent dye and synthesized by Shanghai Sangon Co., Ltd.

The PCR reaction mixture contained 50 ng of genomic DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.33 mM each primer, 0.2 mM dNTP, and 0.2 U Taq DNA polymerase. The PCR program consisted of an initial denaturation at 94 °C for 5 min, followed by 35 cycles consisting of 40 s at 94 °C, 40 s at the annealing temperature (Table 2), 40 s at 72 °C, and a final extension of 7 min at 72 °C.

2.4. Genotyping

The PCR products were mixed with 1:1 with denaturation buffer (98% formamide, 10 mM EDTA, 0.25% bromophenol blue, and 0.25% xylene cyanole), incubated at 95 °C for 5 min, and then immediately chilled on ice. Five microliters of this mixture was then separated on 6% denaturing polyacrylamide (19:1 acrylamide:bisacrylamide) gels using silver staining (Ji et al., 2009). A denatured pBR322 DNA/Msp I molecular weight marker (Tiangen, China) was used as a size standard to identify alleles.

Additionally, PCR amplification of the Amh microsatellite was also done using a fluorescence-labeled Amh primer to further verify the PAGE results. The PCR product was sent to Beijing Liuhe Genomics Inc., for size fragment analysis on an ABI 3730xl DNA analyzer. Chromatograms were scored using Genemarker v1.8 (SoftGenetics LLC: State College, PA, USA).

2.5. Data analysis

Significant differences of survival rate in three developmental stages were tested by analysis of variance with post-hoc

Table 1
Seven pairs of primers for microsatellite amplification.

Locus	Repeat sequences	Primer sequences (5′–3′)	Annealing temperature (°C)	Expected allele size (bp)	Linkage group	GenBank Accession no.
GM385	$(CA)_6CG(CA)_{10}$	F: GAATGAATGATCCCGGCTGTTTGC R: ATCTTCCCGTTGTCCCCATCTTGA	56	289–321	3	BV005434
UNH879	$(AC)_{25}$	F: GCATAAGGTGACTGGCTGGT R: ACAAAGGGGTCCTGCAATTT	52	148–198	23	G68206
GM354	$(CA)_{22}$	F: GACTGTTGTGATTTGTGCT R: GTTCAGGGTTACTGTGTTA	58	252–296	3	BV005419
GM258	$(CA)_{42}$	F: ACAGGTGTGATGAAGCAGAGACT R:GTCAAGATTAGAGATTTCAGGCG	54	142–226	1	BV005380
GM459	$(CA)_{17}$	F: CGGCACATACATCTACTACCT R: TCACCATCAACGCTGAA	51	206–240	1	BV005456
Esr1	$(TG)_5$	F: TCCTTTTATTTGTGAAGTGTCCTCG R: TCAAGCCTGAGGAGTTTGTCTGT	56	143–153	3	NT_167459
Amh	$(AC)_{12}$	F: CAAAGCATTGCCACCAGAGGACC R: TGAACTGCCCTCGCTTGGAAACA	57	270–300	3	EF512167