SHORT REPORT

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Masculinization of the ornamental Siamese fighting fish with oral hormonal administration

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ABSTRACT: Sex control in aquaculture aims to benefit from desirable traits expressed differentially by each sex. In ornamental fish, such manipulation can be profitable, especially in species with high price discrepancy between sexes. This is particularly true for the fancy males of Siamese fighting fish (*Betta splendens* Regan, 1910), which are very popular aquarium pets. Therefore, fry originating from controlled reproduction were fed initially with artemia nauplii from day 4 post-hatch up to day 7 post-hatch. Subsequently, they were orally administered with 17a-methyltestosterone (MT) (via inert feed) in four concentrations (1, 2, 3, and 4 mg/kg of hormone in feed) for eight weeks. The experiment was repeated with fry of the same origin in identical conditions with the exception of a prolonged supply period (from day 4 post-hatch until day 11 post-hatch) of artemia nauplii enriched with red pepper. The results demonstrated that the 3 and 4 mg/kg doses resulted in 100% masculinization and the extended provision period of enriched artemia nauplii increased the survival rate of the offspring. However, mortality rate increased in all hormone treated groups in both experiments, partly due to the prolonged hormone administration period. Therefore, oral administration of low MT doses is effective for the masculinization of Siamese fighting fish.

KEYWORDS: 17a-methyltestosterone, *Betta splendens*, ornamentals, sex-reversal

INTRODUCTION

The Siamese fighting fish (*Betta splendens* Regan, 1910) is a popular freshwater species among aquarium hobbyists [1,2]. Traditionally, Thais breed and sell them for centuries (primarily the males) for their beauty and fighting abilities. However, the species is currently listed as threatened in the wild mainly due to urbanization, industrialization, tourism (including the use of spring water for spas), and agriculture [3,4]. The price of male individuals can be even four times higher than that of the females [5] and accordingly the latter are usually sold en masse at very low prices. Thus the development of reliable and effective techniques to control the gonadal sex of fish and accordingly produce all-male populations in controlled conditions would be an advantage to breeders [6–8].

The main factors affecting the success of sex-reversal are the selection of the appropriate steroid (androgen), the reliability and simplicity of the applied protocol (including doses, feed preparation, and treatment timing related to gonadal development), and the synchronization of offspring production [5,9–11]. All-male production of Siamese fighting fish has been achieved by immersing the fry in 17a-methyltestosterone (MT) solution using concentrations of 100–1000 mg/l at 2, 5, and 8 days post-hatch (dph) [12]. Similarly, norethindrone with doses of 250–1250 mg/l for 3 h on 2nd, 5th, and 8th dph has produced all males [13]. Sex reversal of the same species was also successful with injection of testosterone in adult specimens [14]. Experiments with four different androgens via oral administration demonstrated that fry mortality was far lower in 11-ketotestosterone and androstenedione experimental groups compared to MT and 19-nor-ethynyltestosterone groups [6]. Although sex reversal efforts in the Siamese fighting fish aim to obtain all-male stocks (comprised of both males and masculinized females), it has been also applied to produce all-females using oestrogens or methods involving both direct hormonal treatment and crossings (i.e., indirect sex-reversal) [11,15]. In such cases, the ultimate aim of all-female production was to study their ethology and behaviour [16]. Overall, sex reversal has been applied in 350 teleost species belonging to 23 families and most of them were coral reef-associated species [17].

The aim of the present work was to produce all-male fry of Siamese fighting fish via sex reversal, by using lower doses of MT, compared to that reported in previous experiments involving oral administration.

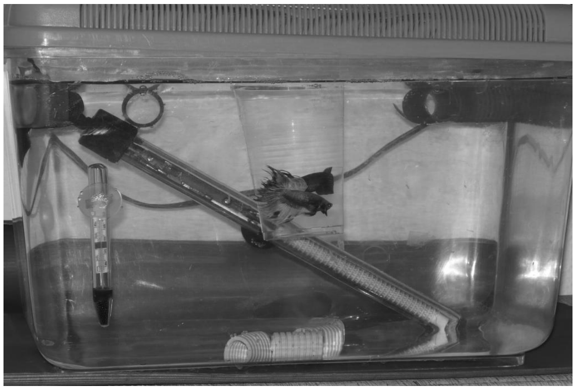

Fig. 1 Breeding aquarium with Siamese fighting fish (floating and sunk flexible spiral plastic tubes are also visible).

MATERIALS AND METHODS

For fry production, 4 mature male spawners were placed in 4 plastic aquaria of 15 l, respectively, along with equal numbers of flexible spiral plastic tubes (Fig. 1) to facilitate the formation of bubble nests and to serve as shelters. The specimens were crowntails, veiltails, and spaidtails of various colours, obtained from the aquarium market. At the third day, one mature female was placed in each aquarium inside a floating transparent plastic glass with holes. In this way, physical separation avoided fighting between the 2 specimens and at the same time enabled visual familiarization and most importantly the release of pheromones. The plastic glasses were removed from the aquaria as soon as the females voluntarily escaped by jumping. The aquaria were visually isolated from each other to avoid aggressiveness by the spawners[18]. Bubble nest formation, spawning, and ova fertilization took place during a period of 2–4 h. Subsequently, the females were removed from the aquaria and males were left for 30 min in order to sustain and prolong bubble formation and facilitate oxygenation of the fertilized eggs. Finally, the eggs were removed along with the bubbles by simply immersing small plastic bowls beneath them. The bowls were then placed inside the same aquaria (at 28 °C), where incubation and hatching took place at 30–36 h post-fertilization. After yolk-sac absorption (72 h at 28 °C), fry were transferred to a new clean plastic bowl to avoid infection.

Sex reversal experiments started on 1 January 2009 (experimental group A) and on 1 May 2009 (experimental group B), respectively. Each group consisted of 400 fry. First feeding in the group A started at 4 dph with artemia nauplii, up to 7 dph. Subsequently, fry were transferred from the 15 l to a 160 l aquarium. In the group B, artemia nauplii enriched with red pepper (Bernaqua NV, Olen, Belgium) were provided from 4 dph up to 11 dph. From 11 dph, fry of group B were also transferred to a 160 l aquarium. In both experimental groups, equal numbers of fry were placed in five small 2 l cages placed in the larger aquarium (four treatment groups and the control groups at stocking densities of 40 fish per litre). Fish were orally treated with inert feeds containing MT (Classic AAF-F until 30 dph and Lucky Star 2 through the end of the experiment at 56 dph) (Taiwan Hung Kuo Industrial Co., Ltd, I-Lan County, Taiwan). Feed preparation protocol (for 300 g of feed for each treatment group) was followed as that of rainbow trout (*Oncorhynchus mykiss*)[19,20] (Table 1). Fish were fed six times per day for equal distribution and consumption of feed by all specimens and to avoid water quality degradation in the aquaria.

Table 1 Experimental design and MT containing feed preparation protocol for Siamese fighting fish masculinization.

Experimental (A, B) and treatment (0–4) groups	Working solution (ml)	90% ethanol (ml)	Final dose of MT in the feed (mg/kg)
A0, B0	0.0	68.9	0
A1, B1	0.1	68.9	1
A2, B2	0.2	68.8	2
A3, B3	0.3	68.7	3
A4, B4	0.4	68.6	4

Sex-reversal was assessed macroscopically under a stereoscope at the end of the each experiment (at the age of 8 weeks), by examining phenotypically each specimen (males were larger and with bigger fins compared to the females[21,22]). The data were statistically analysed with the help of Tukey's test (t-test) and one-way ANOVA (MINITAB 12.0). A probability of $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Experimental group A

Sex reversal and mortality rates in all treatment groups of group A are shown in Fig. 2a. Males reached 100% of the offspring population in treatment groups A2, A3, and A4 and 90% in group A1, compared to 60% in the controls ($P < 0.05$). Mortality rates were higher in groups A3 and A4 compared to groups A1 and A2. However all treatment groups did not show statistically different rates ($P > 0.05$) compared to the controls (A0).

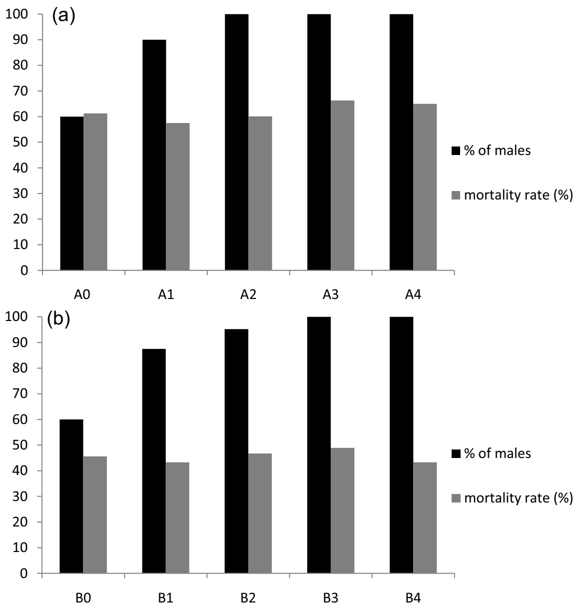

Fig. 2 Proportion of males (%) and mortality rates (%) in different treatment groups of (a) experimental group A and (b) B.

Experimental group B

The proportion of males and the mortality rates in all treatment groups of the experimental group B are presented in Fig. 2b. Sex-reversal in all treatment groups was higher compared to the controls (60%) ($P < 0.05$). In groups B3 and B4, however, males reached 100%. Mortality rates were slightly higher in groups A2 and A3 compared to groups A1 and A4, and the controls, but not at statistically significant level ($P > 0.05$). Mortality rates were significantly lower in the experimental group B compared to the experimental group A, when comparing each treatment group [i.e., ($P < 0.05$) in all the couples A0 vs B0, A1 vs B1, A2 vs B2, etc]. Similar comparison of the proportion of males between groups A and B showed no statistical difference ($P > 0.05$).

Direct hormonal sex-reversal is a valuable tool for sex manipulation and control in fish[23] and legislative obstacles can be transcended in the case of non-edible final products (i.e., ornamental fish). Therefore, wise use of cheap synthetic steroid hormones may increase profit and add significant value when producing monosex offspring of the desired sex. From the current study, it was evident that up to 100% masculinization was possible in the Siamese fighting fish after oral administration of MT (at low doses of 1, 2, 3, and 4 mg/kg) for an 8 week-period. Similar data are limited in the literature. For example, there is a work on Siamese fighting fish masculinization via oral administration of MT, using significantly higher hormonal doses (15, 20, and 50 mg/kg)[6].

During the experiment (group A), mortality increased in all treatment groups, including the controls. Although the hormone is rapidly metabolized[24], mortality could be partly attributed to the prolonged hormonal administration period even in low doses. Nevertheless, weaning problems cannot be excluded, as the duration of artemia nauplii provision affects fry survival and performance[25]. When this period was prolonged only by four days, mortality rate was reduced by 18% (group B). Including highly unsaturated fatty acids (HUFAs) and carotenoids in the feed (via the inclusion of red pepper) also increased the survival rates. HUFAs are valuable for vision, pigmentation, and particularly for the development of brain and nervous system of larvae and fry[26,27]. Red pepper may also play a role as a feeding stimulant, in improving the palatability of feeds and motivating fry towards a more active food searching[28–30]. Nevertheless, aggressiveness was evident in all cages, where fish were concentrated on the surface, as the increased stocking density (40 fry per litre) compromised the offspring welfare. Accordingly, it is suggested that lower stocking densities should be applied in future experimentations.

Low MT concentrations of 3–4 mg/kg are safe and cost-effective for the masculinization of Siamese fighting fish via oral administration. Extended provision period of enriched artemia nauplii, reduced stocking densities and more compressed hormonal administration periods are also necessary to increase the survival rate of the offspring.

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