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Effects of Different Levels of 17α- Methyltestosterone on Growth and Survival of Angelfish (*Pterophyllum scalare* Liechtenstein, 1923) Fry

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Abstract: Angelfish (*Pterophyllum scalare*) fry were immersed in water containing 17-α Methyltestosterone (MT) at the doses of 10, 25, 50, 125, 250 μg L^{-1} water for 30 days. The effects of MT on growth and survival were investigated at an interval of 10 days. Survival of all MT treated groups was significantly lower than control at 20 and 30 th day of the experiment period ($p<0.001$). At the end of the experimental period, the mean length of fry which immersed in MT of 250 μg L^{-1}water were significantly higher than the control ($p<0.05$). However, enhanced growth rate observed in the present study did not compensate the high mortality in practical usage. Anabolic effects of sex steroid on survival and growth of different fish species were also discussed.

Key words: Angelfish, *Pterophyllum scalare*, 17-α methyltestosterone, growth, survival, anabolic effects

INTRODUCTION

Ornamental fish keeping in the Far East dates back to over a thousand years ago, in Europe since the early 17 th century. Since 1950, it has been a popular hobby in both Europe and America and further interest appears to be continuously growing. Only a few hundred of the 4000-5000 fish species currently being kept as pet fish worldwide are very popular. The aquarium trade relies greatly upon fish sourced from the wild involving some degree of habitat destruction and stress to the collected organism. Due to popular demand and pressure on wild resources, culture facilities for ornamental fish, especially the tropical live-bearers, have expanded in countries such as Singapore. Annual international exports of ornamental fish are about 200 US$ million in value, or less than1 percent of total world fish trade. However, the total value of the wholesale ornamental trade is estimated at close to 1 billion US$ and retail trade at about US$3 billion in the United States alone. Asia accounts for more than 50 percent of the world supply of ornamental fish whereas the USA, EEC and Japan are the largest import markets. Singapore is the top ornamental fish exporting country. Singapore re-exports fish originating from neighbouring countries such as Malaysia, Indonesia, Sri Lanka and Thailand[1]. In this commercial situation, angelfish (*Pterophyllum scalare* L.) native to Amazon region of South America, is a cichlid in great demand due to its beauty, reproductive capacity and adaptability to captivity; in consequence, the economic potential of the species is also high. Tropical fish like *P. scalare* is a new species for aquaculture; it is used in hobbies and marketed all over the world.

In aquarium fish external appearance is often markedly different between males and females. In most cases the male fish having more pigmented bodies and more developed fins are preferred over the female fish by the hobbyists. The higher demand to males command a higher price and this price discrepancy could be as high as fourfold over the females. Because the cost of production is similar for both sexes, high discrepancy in market prices makes the culture of all-male populations a highly attractive objective[2].

Sex hormone treatment of embryos leading to functional males of female genotype and/or *Vice versa* serves as a valuable tool for both production of monosex populations in aquaculture industry and understanding the sex determination mechanisms in fishes. In general, estrogen treatments cause feminisation in genetic males and androgen treatments cause masculinisation in genetic females. Direct feminization and masculinization were achieved by androgens and estrogens, respectively in some of the aquarium fishes such as guppy (*Poecilia reticulata*)[3], black molly (*P. sphenops*)[4], poecilid (*Xiphophorus helleri*)[5], the cichlid (*Cichlasoma nigrofasciatum*)[6] and Siamese fighting fish (*Betta splendes*)[7].

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In addition to these effects, steroid hormones can accelerate growth in some fishes. Studies have shown that 17 α- Methyltestesterone (MT) has been shown to be an effective growth promoter in a variety of teleost fish, including coho salmon (*Oncorhyncus kisutch*)[8], chinook salmon (*O. tschawytscha*)[9], masu salmon (*O. masou*)[10], rainbow trout (*O. mykiss*)[11], European eel (*Anguilla rostrata*)[12], carp (*Cyprinus carpio*)[13], blue tilapia (*Oreochromis mossambicus*)[14], Nil tilapia (*O. niloticus*)[15] and grouper (*Epinephelus salmoides*) [16]. In order to better characterize the effects of MT on growth and survival of *P. scalare*, we investigated the effect of different levels of MT for different experimental periods.

MATERIALS AND METHODS

Experimental fish stock, their maintenance and experimental design: The *P. scalare* used in this study were obtained from a commercial aquarium fish production facility of Aqua-Mak Company, Ankara, where the experiments were also carried out.

Fish were kept at 27±2°C under a 14/10 h light/dark cycle. They were fed with a commercial diet (Lansy Dynamic 3 with 42% protein for broodstocks and *Artemia nauplii* for fry) three times a day *ad libitum*. Water quality parameters (O_2, pH, ammonia, nitrite and nitrate) were monitored every fifteen days during the study. A total of 15 aquarium of 60 cmX 30 cmX 40 cm were used for keeping females, rearing fry and establishing hormone treatment units. Two plastic holding tanks of 5 L were placed into a glass aquarium (a total of 12 tanks with 60cmX 30cmX 40cm) in order to provide a certain temperature around the plastic holding tanks for establishing hormone treatment and control units. Yolc sac absorbed fry separated to 5 different hormone treatment regimes. The whole treatment units were filled with clean and aerated tap water before the experiment started. Thermostatically controlled submerged heaters were used to maintain the water temperature at desired level. Aeration was provided to each aquarium through an air pump. All aquariums were cleaned daily by siphoning the uneaten food and excreta and water were then topped up with clean aerated tap water.

A total of 360 fry from 3 females (32.03±1.65 g w/w; 10.70±0.76 cm standard length) were introduced to 5 different level of MT treatments and a control group with two replicates having 30 fish for each. The hormone was administered by immersion to the fry of treatments 1, 2, 3, 4 and 5 (T_1, T_2, T_3, T_4 and T_5) at the doses of 10, 25, 50, 125, 250 μg L^{-1}water and a control (T_0) was maintained. The fry of the control group was immersed in water containing ethanol only. The experiment was lasted 30 days. At an interval of 10 days, 10 fries from each treatment were randomly sampled for measuring length (mm). Dead fry were removed daily from the holding tanks and recorded. After completion of the hormone treatment, they were all measured. Survival was calculated as: (Number of fry at the end of the treatment/Number of fry at the beginning of the treatment) x 100 at an interval of 10 days.

Preparation of hormone: The MT was dissolved in 95% ethanol and a stock solution with a concentration of 500 mg hormone/ ethanol was prepared. 20, 50, 100, 250 and 500 μL^{-1} stock solution per l of water were added to the plastic holding tanks in order to have final doses of 10, 25, 50, 125, 250 μg hormone/l water.

Statistical analysis: Heterogeneity chi-square tests were used between replicates of experimental and control groups for survival. Since no significant differences found in survival of control and experimental groups, the data were pooled in order to increase the number for more meaningful analyses. Survival rates of experimental groups were then compared with control by Fisheries Exact (in case the total number of observations is less or equal to 5), chi-square with Yates correction (in case the total number of observations is less or equal to 25) or chi-square test (the total number of observations is more than 25).

Growth data were expressed as the mean±S.E. Homogeneity of variances was checked with Anderson-Darling test. The significance of differences in growth between control and treated groups were analysed using one-way ANOVA, followed by Tukey's method for multiple comparisons.

A $p<0.05$ was considered as significant. Analyses were performed using Minitab 13 software for windows.

RESULTS

The survival rate of control (T_0) and experimental groups (T_1, T_2, T_3, T_4 and T_5) of fry of hormone treated *P. scalare* varied between 96.67 and70.00% at 10th day of the experiment (Table 1). The survival rates of T_2 (70.00%), T_3 (70.00%) and T_4 (80.00%) were significantly differed from the experimental groups of T_5 (95.00%) and control (96.67%) ($p<0.001$).

The highest survival rate of 95.00 and 95.00% of T_0 were observed at 20th and 30th day of the experiment, respectively. The survival rates of experimental groups were significantly differed from the control at both 20 and 30 th day of the experiment ($p<0.001$).

Table 1: Effects of different levels of 17 α- methyltestesterone (MT) on mean length (mm±standart error) and survival rate (%) of *Pterophyllum scalare* fry at different experimental periods. Common superscipts in the same column indicate means which are not significantly different. ns: Not significant, *** p<0.001

	1. day	10. day		20. day		30. day	
Treatment	Mean length (±SE)	Mean length (±SE)	Survival (%)	Mean length (±SE)	Survival (%)	Mean length (±SE)	Survival (%)
0 (T_0)	5.54±0.14^{a}	6.68±0.19^{a}	96.67	6.68±0.19^{a}	95.00	8.06±0.18^{a}	95.00
10 (T_1)	5.83±0.16^{a}	6.18±0.13^{a}	93.33^{ns}	6.18±0.13^{a}	55.00***	8.20±0.20^{ab}	38.33***
25 (T_2)	5.62±0.11^{a}	6.71±0.10^{a}	70.00***	6.71±0.10^{a}	53.33***	8.90±0.14^{bc}	53.33***
50 (T_3)	5.55±0.17^{a}	6.70±0.18^{a}	70.00***	6.70±0.18^{a}	53.33***	8.10±0.12^{a}	53.33***
125 (T_4)	5.89±0.13^{a}	7.75±0.19^{b}	80.00***	7.75±0.19^{b}	43.33***	8.58±0.20^{abc}	38.33***
250 (T_5)	6.50±0.16^{a}	6.74±0.14^{a}	95.00^{ns}	6.74±0.14^{a}	35.00***	9.44±0.15^{c}	28.33***

The average length of the fry ranged between 5.54±0.14 and 6.50±0.16 mm and no significant differences were found between T_0 and the other treatment groups at the beginning of the experiment (Table 1). The highest mean length was 7.75±0.19 mm in T_4 was significantly differed from the other treatment groups and T_0 at the 10th day of the experiment. The mean length of T_5 (8.98±0.20 mm) was significantly higher than that of the groups of T_0, T_1, T_3 and T_4 ($p<0.05$) but did not differed from T2 ($p>0.05$) at the 20 th day of the experiment. The mean lengths of fry of T_0, T_1, T_3 and T_4 were 8.06±0.18, 8.20±0.20, 8.10±0.12 and 8.58±0.20 mm, respectively and significantly differed from the groups of T_2 and T_5 ($p<0.05$) whose mean length were 8.90±0.14 and 9.44±0.15, respectively, at the end of the experiment. There was no significant differences between the highest mean length of 9.44±0.15mm of T_5, T_2 and T_4 ($p>0.05$).

DISCUSSION

The survival rates of *P. scalare* fry fed on different levels of MT were very low comparing with the control at all experimental periods and they were seem to be duration and dose dependent at least at 30th day of experiment in this study. Low survival of fish treated with steroid hormones was not surprising since in general a treatment involving steroid hormones results in higher mortality of most species. Several studies show estrogens treatment also causes high mortality in mud loach (*Misgurnus mizolepis*)[17], rosy barb (*Barbus conchonius*)[18], Atlantic salmon (*Salmo salar*)[19] and *O. mykiss*[20] perhaps in relation to exhaustion subsequent to enhanced liver metabolism, since it is well known that exposure to estrogenic compounds induces Vitellogenin (Vtg) production, which enlarges the liver[21]. Liver enlargement caused by β-estradiol was showed by histological in *O. mykiss*[20].

Vtg is an egg yolk protein precursor normally produced in the liver of female oviparous vertebrates in response to circulation endogenous estrogen. Once produced in the liver, travels in the bloodstream to the ovary where it is taken up and modified by developing eggs. Vtg is normally undetectable in the plasma of immature female and male animals because they lack circulating estrogen, although the Vtg gene in these animals can be induced by estrogen treatment. For this reason, Vtg production in male fish has become a widely used indicator of exposure to exogenous estrogen or estrogen mimics in the aquatic environment. Several studies have linked high plasma Vtg concentration in males of aquatic species with increased mortality[22]. Low survival effect of MT was reported in adult fathead minnows (*Pimephales promelas*) by Ankley *et al.*[23]. In their study adult male and female *P. promelas* were exposed to MT of 120 μg L^{-1} and 1700 μg L^{-1} for 12 days. At these high concentrations only 20% of the fish survived. MT strongly induced Vtg synthesis in both sexes. This result was attributed to the aromatization (the catalysing procedure converting androgens to estrogen) of the MT by the minnows and subsequent stimulation of the 17 α estradiol (E2) receptor which stimulates producing Vtg. Dose and duration dependent high mortality was reported by George and Pandian[4] in *P. sphenops* fed by MT with different concentrations from 10 to 400 mg kg^{-1} diet for 15, 30, 45 days. The survival at sexually maturity of individuals treated for 30 days declined steeply from 56 to 2%, when MT dose was increased from 200 to 300 mg kg^{-1} diet. The high mortality seen in this study could be resulted from aromatization of MT to estrogen stimulating high Vtg production which damage liver of fish. This result may show that estrogen treatment of *P. scalare* may also cause high mortality. In this regard, further research need to clarify this assumption.

Our result indicate that treatment with a concentration of 125 and 250 μg L^{-1}MT in water had positive growth effect on *P. scalare* for 20 and 30 day of experiment period, respectively. Anabolic steroids, both androgen and estrogen enhance the growth and feed conversion efficiency when administered at optimal level in fish[24]. Androgen induced growth enhancement were reported by several authors in several fish species (see introduction). Growth Hormone (GH) stimulates

growth directly by stimulating cell differentiation and indirectly by inducing the production and release of a mitogen, Insulin-like Growth Factor-I (IGF-I), which is produced by both liver and most peripheral tissues[25]. In mammals and teleost, gonadal steroids have been shown to affect growth primarily by interacting with the endogenous growth regulators including GH and IGF-I and therefore, somatic growth is regulated primarily through the GH/IGF-I axis[25]. Riley *et al.*[26] were reported that MT fed *O. mossambicus* in freshwater and seawater showed improved growth rate comparing with controls. MT treated seawater fish had also significantly higher levels of GH mRNA levels than all the other groups indicating that MT is involved in regulating GH cell activity and subsequently growth. However, an opposite response has been described also in Sea bass (*Dicentrarchus labrax*)[27] and *C. carpio*[28]. Also many authors have not chosen to undertake long-term studies on the growth rate of androgen fed fish. In *P. reticulata*, the dose of 50 mg kg^{-1} diet promoted the maximum anabolic growth enhancement in juveniles younger than 6 months. However the relative growth began to diminish at the age 9 and 18 months[29]. The magnitude of the response to MT depends on many factors including age, size, developmental stage, temperature, salinity, dietary factors, treatment duration, season, method of application[30] and also species. On the other hand enhanced growth rate observed in the present study did not compensate the high mortality in practical usage. To our knowledge, the present study is the first report about the effect of MT on survival and growth of *P. scalare*. Thus, there is a need for determining the minimum dose ensuring low mortality before searching the positive effect of sex steroids on growth of *P. scalare* fry on both fry and adult stages.

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