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EFFECT OF DIETARY THYROXINE SUPPLEMENTATION TO BROOD FISH ON EARLY LARVAL DEVELOPMENT OF CLIMBING PERCH, *ANABAS TESTUDINEUS*

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Effect of dietary thyroxine (T_4) supplementation to *Anabas testudineus* brood fish on growth of their larvae were evaluated. The brood fish were divided into four groups - one group was fed with control diet and rest three groups were fed with 2 mg T_4/kg diet, 5 mg T_4/kg diet and 10 mg T_4/kg diet, respectively for a period of 4 weeks. After 4 weeks, the fish were induced to breed using Ovaprim and the larvae were reared separately for a period of two weeks. The results indicated that larvae of T_4 treated spawners exhibited significant increase in growth and also tended to show better survival compared to larvae of control group spawners. When larvae of control and T_4 treated spawners were reared in thyroid hormone containing rearing medium (0.05 ppm), it was indicated that T_4 at 0.05 ppm accelerated growth in larvae of control spawners and consistently, the same did not induce growth increment in the case of T_4 treated spawners larvae. Hence, it was considered that in the case of T_4 treated spawners, there might be transfer of thyroid hormone from mother to the oocytes and subsequently to the larvae and further treatment through immersion might have resulted in over dose of thyroid hormone leading to thyrotoxicosis and thereby growth retardation.

INTRODUCTION

Fish larvae are physiologically immature with little or no capacity to produce certain hormones and are dependent to a greater or lesser extent on exogenous sources (mother and/ or live food) for supply of regulatory factors (Greenbalt *et al.*, 1989; Brown *et al.*, 1988, 1989; Ayoson and Lam, 1991; Lam, 1994; Kang and Chang, 2004). Hormones are passed on to eggs by brood fish and store of maternal hormones may fill the regulatory needs of fish larvae for growth, development and other physiological functions prior to the functional development of their own endocrine glands (Inui and Miwa, 1985; Brown *et al.*, 1988, 1989; Brown and Bern, 1989; Tagawa and Hirano, 1987; Tagawa *et al.*, 1990a,b; Reddy and Lam, 1992a,b; Kang and Chang, 2004).

Thyroid hormones (thyroxine, T_4 and triiodothyronine, T_3) are present in fish eggs and in most cases, the levels decrease as development proceeds until the onset of endogenous thyroid hormone production which usually occurs before or at around yolk

sac resorption (Ingibjörg *et al.*, 2006). Enhancement of T_4/T_3 levels in newly hatched larvae through immersion or maternal injection has been shown to promote growth, development and/or survival in several fish species (Lam, 1980; 1985; Nacario, 1983; Lam and Sharma, 1985; Lam *et al.*, 1985; Reddy and Lam, 1987; Miwa and Inui, 1987; Yamano *et al.*, 1991; Miwa *et al.*, 1992; Reddy and Lam, 1992). Routine applications of hormones to fish by injection or immersion are uneconomical and also not feasible in aquaculture management point of view. Hence, oral administration is potentially more practical. In the present study, attempt was made to evaluate the effect of incorporation of thyroxine (T_4) in the diet of broodstock on growth and survival of the larvae after rearing for a period of two weeks.

MATERIAL AND METHODS

Forty-two advanced maturing *Anabas testudineus* (average length 11.49±0.32 cm and weight 31.25±1.63 g) were used in the present experiment. The fish were collected from the local fish market of Berhampur, Orissa. After collection, the fish were acclimatized to the laboratory conditions and gradually weaned to an artificial (moist) feed. The moist feed was prepared from boiled chicken viscera, fish meal, groundnut oilcake, rice bran, wheat flour and vitamin and mineral mixture at 25, 25, 25, 20, 04 and 1% respectively. Using the moist feed, three experimental diets were prepared by incorporating thyroxine (T_4) in three different concentrations *i.e.* 2, 5 and 10 mg/kg of diet separately. Eltroxin (Glaxo, India) tablets, each containing 0.1 mg of L-thyroxine sodium, was used for incorporation of T_4 in the diet. The fish were divided into 4 groups containing 13 numbers of fish (5 females and 8 males), out of which 3 groups were fed with experimental diets and one group was fed with control diet, without thyroxine, for a period of four weeks and were maintained in separate aquaria containing 40 liter of water. Due to less numbers of brood fish available, replications were not maintained.

After four weeks of rearing, 4 females and 6 males (in fully ripe condition) were selected from each group and induced to breed. Each female fish was injected with 0.2 ml and each male fish was injected with 0.1 ml of Ovaprim. After injection, each group of fish was kept separately and after spawning, the fertilized eggs from each group were kept separately in flow-through system for hatching. For larval rearing, 100 numbers of yolk sac larvae were collected from each group and divided into two sub-groups containing 50 larvae each. Out of two sub-groups, one group was reared in normal water whereas other group was reared in water containing thyroxine at concentrations of 0.05 ppm. This optimum dose was decided from the results of the experiments conducted earlier by the authors. Eltroxin (Glaxo) tablets each containing 0.1 mg L- thyroxine sodium was used for preparing the thyroxine (T_4) media. The larvae were fed with *Artemia* nauplii. The numbers of *Artemia* nauplii in the tank were increased depending on the requirement.

After two weeks, the larvae were sampled and the number of surviving larvae were counted and final length and weight were recorded.

RESULTS AND DISCUSSION

Results of the experimental studies are presented in the Table 1. After 14 days of rearing, larvae of T_4 treated spawners were found to be comparatively larger than the larvae of control spawners. Between the T_4 treated spawners, the larvae of spawners treated with 5 mg T_4 were comparatively larger. The weight of the larvae of spawners treated with 5 mg T_4 were also higher and so also the survival rate, which indicated that there exist a dose dependent response *i.e.* after an optimal level, the increase of hormone dose resulted in a decrease in growth as the larvae of 2 mg and 5 mg T_4 treated brood fish maintained a positive growth trend but the larvae of 10 mg T_4 treated brood fish exhibited a negative trend in growth. These findings were consistent with results of Ayson and Lam (1993) who had reported that in rabbit fish, larvae of T4 treated spawners were longer than the control and exposure of larvae to a higher concentration of hormone had resulted in retardation of growth *i.e.* larvae of spawners treated with 100 µg T_4/g body weight were comparatively shorter than the larvae of 10 µg T_4/g body weight treated spawners.

Table 1. Effects of dietary thyroxine treatment of broodstock and immersion on growth and survival of *Anabas testudineus* larvae

Treatment	Larval Growth			
	1 day	15 day		
	Total length (mm)	Total length (mm)	Average weight (mg)	Survival (%)
C	2.32±0.15	11.92±1.0	61	69
DT-2	2.41±0.07	17.66±1.43	66	72
DT-5	2.45±0.22	18.46±1.24	77	80
DT-10	2.58±0.22	17.61±1.03	60	72
C+0.05 ppm	2.32±0.15	16.05±0.63	105	78
DT-2+0.05 ppm	2.41±0.07	14.67±0.85	31	70
DT-5+0.05 ppm	2.45±0.22	14.30±0.62	28	70
DT-10+0.05 ppm	2.58±0.22	14.0±1.4	26	70

C: Control; DT-2: Broodstock fed with 2 mgT_4/kg diet; DT-5: Broodstock fed with 5 mgT_4/kg diet; DT-10: Broodstock fed with 10 mgT_4/kg diet; 0.05 ppm: rearing medium containing 0.05 ppm T_4.

It is interesting to note here that when larvae of control group of spawners were treated with 0.05 ppm T_4 (through immersion), it resulted in acceleration of growth but when larvae of T_4 treated spawners were treated with 0.05 ppm T_4 through immersion, consistently in all the groups, it resulted in growth retardation. Hence, it was assumed

that hormones fed to the broodfish might have passed on the larvae through eggs. In addition, the immersion treatment might have resulted in elevation of thyroid hormone level in the larvae. Further, there might be thyroxinogenesis (endogenous thyroid hormone synthesis). As a result, all the three sources (maternal transfer + uptake through immersion + thyroxinogenesis) might have resulted in overdose of thyroid hormone and that might have manifested in the larvae by growth retardation. The deleterious effects of such growth inhibition, abnormal development at higher doses of T_4 also have been reported in other teleost larvae (Dales and Hoar, 1954; Honma and Mirakawa, 1955; Lam, 1980; Nacario, 1983; Lam and Sharna, 1985; Reddy and Lam, 1992a,b; Nugegoda *et al.*, 1994). On the other hand, in the control group of larvae, treatment through immersion (only) might have resulted in the optimum hormone level thereby resulting in growth acceleration.

Hence, from the present study, it is considered that thyroid hormones administered to parents prior to spawning through diet may improve the larval quality and reduce the larval rearing period by accelerating growth and development by enhancing the hormonal level in the early physiologically immature stages when the larvae have no capacity to produce hormones. In addition, no further treatment through immersion is required to enhance the larval growth once the broodstock are treated with dietary thyroid hormone at appropriate doses before spawning. Since thyroid hormone treatment of larvae immersion is not practical on commercial scale, broodstock manipulation may offer a great potential for development as a practicable method for improving larval quality in aquaculture.

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