Egg production and larval rearing of orange-spotted grouper (*Epinephelus coioides*) using reared broodstocks in Hormozgan Province, Iran

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Abstract

Egg production of cultivated broodstocks of orange-spotted grouper, *Epinephelus coioides* and larvae rearing were surveyed in Iran from 2008 to 2012. Broodstocks were obtained by rearing of 7 wild juveniles (587.57±132.63 g body weight) who were caught from the north of Persian Gulf in October 2008 and reared to December 2012. Captured juvenile fishes were fed using defrosted trash fish at a rate of 4-6 percent of body weight per day. Four-year-old fishes spawned spontaneously, when weight of females and males average were about 7020±1277 g and 5128±253 g, respectively. Effects of four temperature ranges (23-24, 26-27, 28-29 and 31-32°C) and three tank size (40L, 300L and 2400L) on hatching and survivle rates were examined. Eggs were stocked as a density of 25 Eggs L^{-1}. Effects of tank size on hatching rate and survival of larvae were assessed at 1, 5, 10, 20 and 30 days after hatching (DAH). The optimal temperature range for hatching was 28-29°C. No difference was observed among hatching rates of fish within the various tank sizes. Survival rates of larvae in 2400L fiber glass tanks until 30 days after hatching were significantly higher than the other tanks. Results indicate this species can be reproduced and reared in large numbers under normal rearing conditions and carefully controlled temperature.

Keywords: Orange-spotted grouper, Eggs production, Larvae rearing, *Epinephelus coioides*, Tank size

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Introduction

Marine species aquaculture such as shrimp and marine fishes has been regarded as an important contributor to the economies of southern provinces of Iran. The technique of artificial reproduction and seed production of some species of marine fish such as, yellow fin sea bream (*Acanthopagrus latus*) and Sobaity sea bream (*sparidentex hasta*) has almost been expanded and some species such as Rabbit fish (*siganus sutor*) and grouper (*Epinephelus coioides*) are still at the embryo stage (Fourooghifard and Daghooghi, 2006; Abbassi *et al.*, 2008; Bahmani *et al*., 2009, Fourooghifard e*t al*., 2009).

Groupers are classified into 14 genera of the subfamily Epinephelinae, which comprises at least half the approximately 449 species in the family *Serranidae*. They are found throughout most warm and temperate marine regions. Serranids are highly valued for food, and both small and large species are kept in aquaria (Tucker, 1999).

Groupers are highly valued for the quality of their flesh, and high market prices. They are the most intensively exploited group in the fish trade, and the high prices paid by exporters to local fishermen mean that target species may be heavily over-fished (Morris *et al.,* 2000).

E. coioides occurs in the western Indian Ocean from the southern Red Sea to Natal and east to the western Pacific where it is distributed from Ryukyu Islands to New South Wales. It ranges east into Oceania only to Palau in the Northern Hemisphere and Fiji in the Southern. Orange-spotted groupers inhabit turbid coastal reefs and are often found in brackish water over mud and rubble. Juveniles are common in the shallow waters of estuaries over sand, mud and gravel and among mangroves, feeding on small fish, shrimp, and crabs. *E. coioides* are Eurythermal and Euryhaline (FAO, 2012).

E. coioides like other serranid species are protogynous hermaphrodites. They first mature as females at around 3-4 kg body weight. Some of the fastest growing females transform into males when they reach more than 6 kg body weight. Because of the difficulty in obtaining mature males, studies to develop methods to induce sex-inversion in juveniles and adults were undertaken (Tan-Fermin *et al.,* 1994)

The major spawning period is from March to June. Females mature at 250-300 mm TL at an age of 2-3 years; sexual transition occurs at a total length of 550-750 mm. Fecundity estimates varied from 850 186 ova in a 350 mm TL fish to 2 904 912 ova for one of 620 mm TL. Eggs are pelagic; best survival of larvae is attained at 30°C and 30‰. Successful artificial propagation of *E. coioides* has been reported in Malaysia. They probably spawn during restricted periods and form aggregations when doing so and the eggs and early larvae are probably pelagic (FAO, 2012). Grouper larvae are stocked at relatively

high density: 20–30 per liter (Ruangpanit, 1993; Duray *et al*., 1996)

In general, the main factors that determine larval mortality are biotic factors (For example food, disease, parasitism and predation) and abiotic factors (for example oxygen, pH, salinity, toxic substances and temperature) (Kamler, 1992).

Material and methods

This study was conducted at the Persian Gulf and Oman Sea Ecological Research Center, which is located in Hormozgan Province. The project was conducted from March to June 2012.

Broodstocks were obtained by rearing wild juveniles that captured from 2007 to 2008 and reared until the year 2012. Fishes were reared for a period of four years until they matured. Captured juvenile fish were stocked in fiberglass tanks at a density of 6-4 kgm^{-3} and were fed defrosted trash fish at a rate of 4-6 percent of body weight per day. Fish were individually tagged by cutting off dorsal fin spins for identification of their growth rate and maturation and reared for a period of 4 years until they matured. In the 3rd year of culture, a group of tagged fish was transferred into a 5 m^3 fiberglass tank.

Before the reproduction season (February), a running water and aeration system were designed to get eggs moved forward in an egg collector. An easy system was designed to collect the floating eggs (Table 1) (Figs. 1-3).

Eggs were collected during February till March 2012. The diameter of eggs was measured by Stereomicroscope equipped with a microcomputer. Diameter of 30 eggs was measured.

In order to determine the best water temperature, for eggs incubation , four various ranges of temperature included 23-24, 26-27, 28-29°C and 31-32°C were compared. Experiments were conducted in 300 L polyethylene tanks which contained 200 L water. Eggs were stocked as a density of 25 eggs per liter**.** Effects of various tank sizes (40 L aquarium, 300 L polyethylene tanks and 2400 L fiber glass tanks) on hatching and survival rates of larvae were surveyed on days: 1, 5, 10, 20 and 30 DAH. All experiments were performed with 3 replications. Hatching rate were obtained by counting the larvae after 1 DAH[1]. Survival rates were obtained by counting the number of survival larvae per liter. Larvae were fed by *Nannochloropsis* and sieved rotifer (45-80 μm) *Brachionus plicatilis* at a density in sequence, 500000 cell/mL and 15-20 ind mL^{-1}.

Results

4 years old, orange-spotted grouper spawned spontaneously, when average weight of females and males was about 5.360±0.253 kg and 7.020 ± 1.277 kg, respectively in 2012, Eggs were collected from February to March 2012. The fertilized egg of *E. coioides* was round with its shell membrane, measuring about 857.70 mm (n=30) in diameter (Fig. 4).

[1.] Days After Hatching

Table 1 : Data of the initial and final biometry of grouper (*Epinephelus coioides*) reared in fiberglass tanks.

Number	Initial data (September of 2008)			Final data (July of 2012)			gender
	Total length (Cm)	Body height (Cm)	Body weight (g)	Total length (Cm)	Body height (Cm)	Body weight (g)	
1	36	9	820	77	20.5	7855	male
2	37	9	850	72	19	6825	male
3	29	8	440	68	18	6380	male
4	31	8	495	65	18	5250	female
5	33	8	595	66	18	5360	female
6	28	7.5	400	60	17	4800	female
7	28	8	450	59	17	4850	female

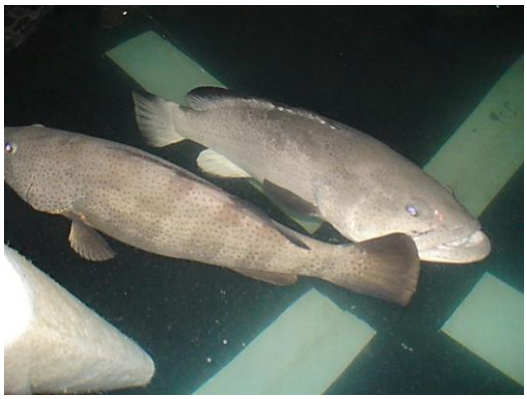

Figure 1: Broodstocks of *Epinephelus coioides* in spawning tank.

Figure 2: Running water and aeration system to facilitate movement of eggs to egg collector.

Figure 3: Designed system to collect the floating eggs of *Epinephelus coioides.*

Most of the eggs held embryos in themselves. Eggs of *E. coioides* hatched at temperatures above 25°C. According to results, the average length of newly hatched larvae (1 day larvae) was about 2207.86 ± 54.04 µm and they had no pigment in their eyes. Larvae started exogenous feeding 2 or 3 days after hatching (Fig. 5).

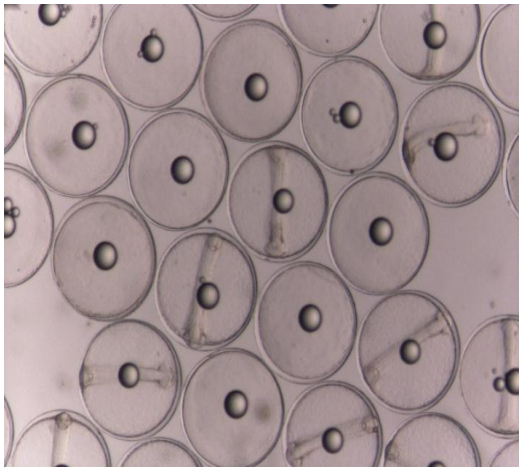

Figure 4: Fertilized eggs of *Epinephelus coioides*.

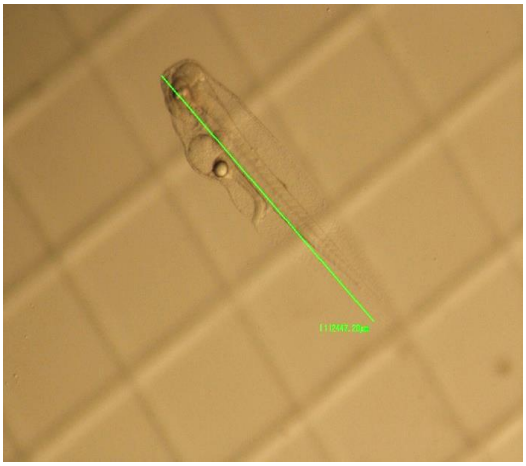

Figure 5: Newly hatched larvae (1 day larvae) *Epinephelus coioides.*

Results of comparison between various levels of water temperature on hatching rate of *E. coioides* indicated significant differences between them (p<0.05). At the temperature range of 23 - 24°C the hatching rate was 0. The best range of temperature for hatching was 28-29°C (Fig. 6)

Results also indicated that there were no differences between the hatching rate of eggs in different tank sizes (p> 0.05) (Fig. 7).

Survival rates of larvae until 30 DAH were better in bigger tanks than in the others. There were significant differences between survival rates of larvae in aquaria, 300 L polyethylene tanks and 2400 L fiber glass tanks (p<0.05) (Fig. 7).

According to the results, there was a huge decrease of larval density on days 5 and 20 (Fig. 8).

After 30 days of rearing the best survival rate was obtained in 2400 L fiberglass tanks with a rate of 16±9.94%. In all treatments metamorphosis of larvae was observed on day 7 after hatching (Fig. 9).

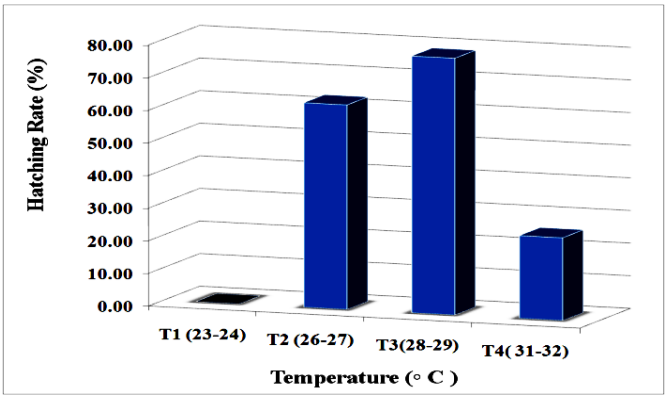

Figure 6: Hatching rate of *Epinephelus coioides* in various ranges of Temperature.

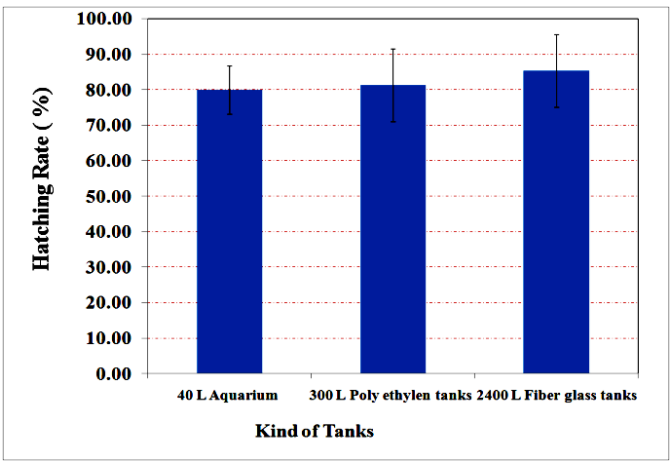

Figure 7: Hatching rate of *Epinephelus coioides in* various tank sizes .

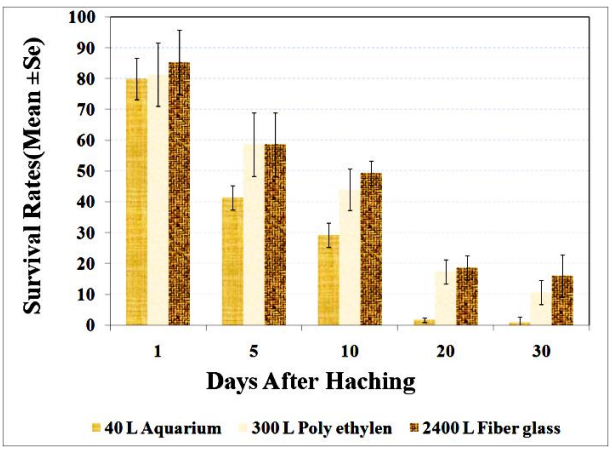

Figure 8: Survival rate of *Epinephelus coioides* in various tank sizes.

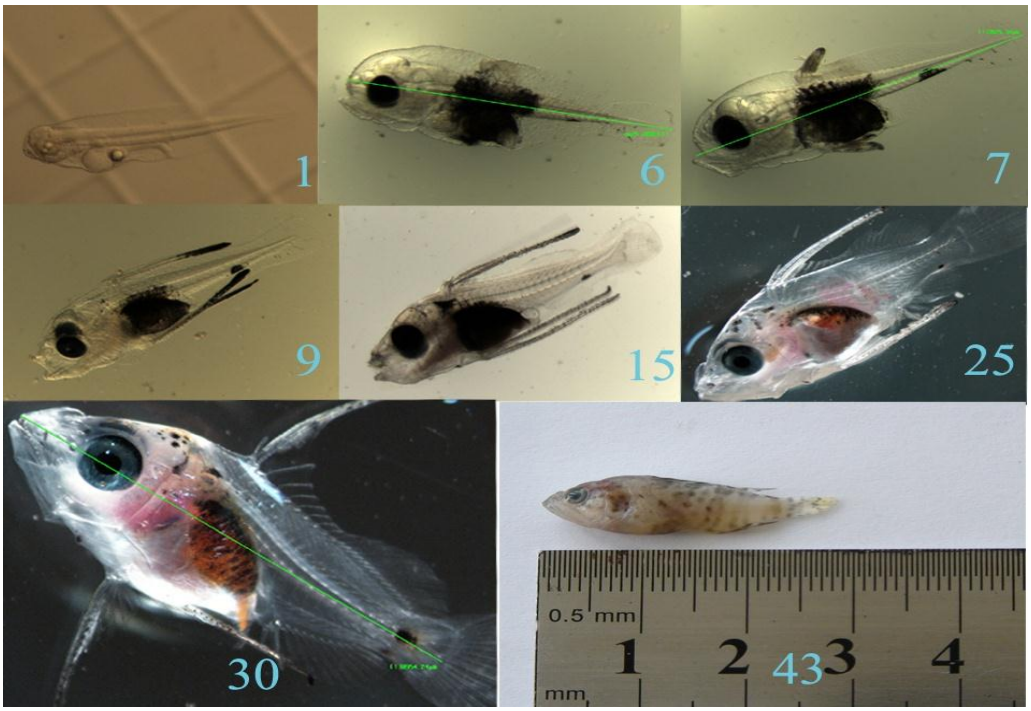

Figure 9: Metamorphesis of *Epinephelus coioides* larvae (numbers indicate the DAH).

Discussion

Results of natural spawning of *E. coioides* revealed that sex transition of this species in captive can occur when their body weight is about 6 kg and they are 5 years old.

Available report indicates that sex transition of *E. coioides* in nature occurs when they are 7 years old (Dehghani *et al.*, 2001).

Results of this study revealed that spontaneous spawning of *E. coioides* can occur frequently without the use of any hormone under natural light inside a covered saloon.

Voluntary spawning of captive groupers has occurred mostly with well-fed, uncrowded fish during the natural spawning season under conditions of ambient temperature and partial or total natural light (Tucker, 1999). Results of comparison between various levels of water temperature on hatching rate of *E. coioides* indicate that the best range of temperature for hatching was 28 - 29°C (Fig.6).

Sugama (2004) reported that growth of humpback grouper larvae, *Cromileptes altivelis*, increased with increasing water temperature. According to this report the highest survival (48.11%) was found for larvae reared at 28°C that was much higher, compared with the survival of larvae reared at 31°C, which was only 4.77%.

Akatsu *et al.* (1983) reported that for brown spotted grouper *Epinephelus tauvina* reared for 12 days at different water temperatures, total length of larvae reared at high temperature (32°C) was the highest (6.5 mm)

compared with larvae reared at 23°C (4.1 mm).

According to the observations and data obtained of larvae counting, there was a huge decrease in larval density on days 5 and 20 (Fig. 8).

High mortality was associated with the commencement of exogenous feeding. This mortality may be associated with the provision of live prey organisms of unsuitable size and nutritional composition, but even when 'suitable' prey types were used, there is generally high mortality at this stage (Ordonio-Aguilar *et al*., 1995; Duray *et al*., 1997).

Several mortality syndromes have been described for grouper larvae. A commonly reported mortality syndrome is the 'shock syndrome' that occurs in late stage larvae from about D25 (Lim, 1993; Duray *et al*., 1997). This problem may be related to nutritional deficiencies in the live prey organisms used to feed the larvae, since shock syndrome is symptomatic of low levels of HUFAs in the diet (Cowey and Sargent, 1972).

Survival rates of larvae until 30 DAH was better in bigger tanks than in the others. Results revealed that by increasing of tanks size the survival rate of larvae increase. After 30 days of rearing the best survival rate was obtained in 2400 L fiberglass tanks at a rate of 16 ± 9.94%.

Tank size, shape and color can affect the survival of grouper larvae. *E. coioides* larvae cultured in 3 m^3 tanks demonstrated a better survival rate (19.8%) on Day 24 compared with only 7.4% for those in 0.5 m^3 tanks on D21 (Duray *et al.*, 1997).

Overall results revealed that the survival rate of grouper larvae can be improved by the use of large tanks and controlling water temperature.

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