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Effects of Incubation and Spawning Water Temperature, and Salinity on Egg Development of the Orange-Spotted Grouper *(Epinephelus coioides, Serranidae)*

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Abstract

Responses of orange-spotted grouper (*Epinephelus coioides*) eggs to various incubation water temperatures and salinities were measured in terms of incubation period and hatchability. Eggs spawned naturally by the grouper in captivity were used for this study.

Time from spawning to hatching was inversely related to incubation temperature. Time to hatching was 32.4, 24.0, 19.5, 16.5 and 15.8 h at 23, 26, 29, 32 and 35°C, respectively. Egg development at each incubation temperature proceeded at a uniform rate.

The lower limiting incubation temperature for 50% viable hatching of the fertilized eggs spawned at 23-27°C was estimated at 22.1-23.2°C. The upper range was estimated to be 30.1-31.0°C. No eggs hatched at the incubation temperature of 20°C, and the few eggs hatched at 34°C resulted in 100% abnormal larvae.

The viable hatching rate was high in incubation salinities of 17-47 ppt. No egg hatched at 0 ppt salinity. The rate of abnormal larvae was high in 7 and 57 ppt incubation salinity. Differ-ences in hatching time at incubation salinities of 17-47 ppt were insignificant.

Introduction

The orange-spotted grouper, *Epinephelus coioides*, has been referred to as *E. suillus* by many scientists (Randall 1987; Kohno et al. 1990; Doi et al. 1991). Recently, Randall and Heemstra (1991) indicated that *E. suillus* was a synonym of *E. coioides*.

This grouper occurs in the western Indian Ocean from the southern Red Sea to Natal and east to the western Pacific, and the Mediterranean Sea (Randall and Heemstra 1991). In the Arabian Gulf, it is the dominant large grouper and the most important food fish. The greasy grouper, *E. tauvina*, was not known from the Arabian Gulf, and *E. coioides* has been misidentified as *E. tauvina* (Randall 1987).

Water temperature and salinity are factors which have a major effect on the development of marine teleosts (Blaxter 1969). Their effects on *E. coioides* larval survival and growth were reported for Kuwaiti waters (species referred to as *E. tauvina* by Akatsu et al. 1983). In this paper, the effects of temperature and salinity on egg development of the grouper are explored.

Materials and Methods

Spawners and Eggs

E. coioides spawners were collected from Bahraini waters, and cultured for about 3 years in concrete tanks at the National Mariculture Center (NAMAC). By 11 January 1993, the broodstock consisted of two males (14-15 kg·fish^{-1}), 11 females (8-13 kg·fish^{-1}) and three sexually undetermined fish (10-13 kg·fish^{-1}). Well water at 27-28°C and 47-48 ppt salinity was supplied to the tank.

Spawning occurred naturally in the 120 m^3 concrete tank. Eggs were collected through an overflow system into a fine-meshed net suspended soon after spawning. Time at spawning was estimated by: 1) fish spawning behavior as a pair of male and female jump from the water surface at spawning, and 2) time of finding eggs. Egg fertilization rate was 37.5-82.4%. Fertilized eggs used for experiments were at the 2-8 cell stage. During the study period (16 February-24 June 1993), water temperature in the spawning tank was raised from 22.8 to 27.6°C. Salinity remained at 47-48 ppt.

Temperature Experiment

The experiment was carried out in a wet laboratory in NAMAC with air temperature controlled at 20°C. Well water at 48 ppt salinity was used. The water was first aerated for 1-2 h so that dissolved oxygen was at saturation levels. Egg incubation temperature was maintained by water baths equipped with thermostatically controlled heaters. The experiment consisted of three tests: 1) embryonic development, 2) incubation period, and 3) hatchability.

EMBRYONIC DEVELOPMENT

Time from spawning to various embryonic stages was recorded at different incubation temperatures of 23, 26 29 and 32°C. Eggs were obtained on 20-26 February when spawning temperature was 23.1-24.2°C. Approximately 1,500 eggs each were stocked into 3-l beakers with 2.5 l of water set at the desired temperature ($\pm$0.1°C). Each beaker was covered with a plastic sheet to minimize water evaporation. During the incubation period, water was moderately aerated but not changed. Dead eggs were removed by pipette whenever they were found. Ten to 30 eggs were sampled hourly and their development was examined by microscope. These eggs were not replaced. When hatched larvae were found, they were removed and counted. Determination of egg developmental stage was based on the stage of most eggs in a single sample. Hatching time was defined as the time when 50% hatching occurred.

INCUBATION PERIOD

Time from spawning to hatching at various incubation temperatures between 20 to 35°C (in 3°C intervals) was recorded. Batches of 200-300 eggs, spawned at 23.4°C (1 March), were stocked in each of six 1-l beakers with 0.7 l of water set at the desired temperature. Egg incubation methods followed those in the above test, however, aeration was not provided and dead eggs were not removed. The water temperature remained within $\pm$0.3°C of the desired temperature. Eggs were observed every hour (starting from spawning time). Whenever hatched larvae were found, they were removed and counted. Time to 50% hatching was estimated by linear interpolation following Kashiwagi et al. (1984). This procedure was repeated with the eggs spawned at 23.9°C (4 March).

HATCHABILITY

Hatching rates and occurrence of abnormal larvae were recorded for eggs incubated at 20-34°C (in 2°C intervals). Spawning temperatures were at 23, 25 and 27°C ($\pm$0.2°C, refer to Table 2). Batches of 200-300 eggs were stocked in each of 16 1-l beakers with 0.7 l water with temperature similar to that in the spawning tank. Two beakers were placed in a water bath set at each incubation temperature. Water temperature in the beakers reached the desired incubation temperature within 30 minutes, and the temperature was maintained within $\pm$0.4°C of the desired incubation temperature. Egg incubation methods followed those of the hatching speed test. After hatching, the number of total larvae (TL), abnormal larvae including dead larvae (AL), and dead eggs (DE) were counted. Total hatching rates (TH), viable hatching rates (VH) and abnormal larvae rates (ALR) of the fertilized eggs were calculated as follows.

TH(%) = {TL/(TL+DE) x 100}/(FR/100)
VH(%) = {(TL-AL)/(TL+DE) x 100}/(FR/100)
ALR(%) = AL/TL x 100
where FR = fertilization rate of eggs used.

Salinity Experiment

In this experiment, two tests were conducted: 1) incubation period and 2) hatchability. The experiment protocol was the same as that described in the temperature experiment. Incubation salinities examined in both tests were at 0, 7, 17, 27, 37, 47 and 57 ppt. To obtain the desired salinities, well water (47 ppt) was diluted with freshwater aerated for one day, or mixed with synthetic sea salt. Egg incubation temperature was adjusted to the water temperature of the spawning tank by a water bath ($\pm$0.1°C). To avoid eggs piling up at the bottom of the beakers in low salinity water, egg stocking density was lower (100 eggs•beaker^{-1}) than in the temperature experiment. For the incubation period test, eggs spawned at 25.2°C (24 April) and 27.4°C (17 June) were used. The hatchability test was conducted twice using eggs spawned at 27.4°C (21 and 24 June).

Results

Temperature Experiment

EMBRYONIC DEVELOPMENT

Development times from spawning to various embryonic stages under varying incubation temperatures are summarized in Table 1. As incubation temperature increased, development time decreased. The relationship between development time to each stage (T hours) and incubation temperature (23°C < t < 32°C) can be expressed by the equation $T = a + bt + ct^2$. Time and temperature were compared for each development stage (Fig. 1). All lines in Fig. 1 are essentially parallel to each other. This indicates that for each incubation temperature, the rate of embryonic development up to hatching is proportional.

Table 1. The attainment time (h) to various embryonic stages from spawning under different incubation temperatures in *Epinephelus coioides* eggs.* Relationships between time and incubation temperature is indicated by the equation.

Embryonic stage	Incubation temperature (°C)				Equation**
	23	26	29	32	
Morula	4	3	2	2	$T = 29.861 - 1.761t + 0.028t^2$
	(3-4)	(2-4)	(2-3)	(2-3)	(r = 0.991)
Blastrula	6	5	4	3	$T = 13.667 - 0.333t$
	(5-7)	(4-5)	(3-4)	(3-4)	(r = 1.000)
Gastrula	9	6	5	4	$T = 62.056 - 3.589t + 0.056t^2$
	(8-11)	(5-7)	(4-5)	(4-5)	(r = 0.993)
Appearance of embryo	13	10	8	7	$T = 69.222 - 3.722t + 0.056t^2$
	(12-15)	(9-11)	(7-9)	(6-7)	(r = 1.000)
Appearance of Kupffer's vesicle	16	12	10	9	$T = 94.917 - 5.350t + 0.083t^2$
	(15-17)	(11-13)	(10-11)	(9)	(r = 0.999)
Formation of lens	23	17	14	13	$T = 150.472 - 8.739t + 0.139t^2$
	(23-25)	(16-17)	(14-15)	(12-13)	(r = 0.999)
Commencement of heart beat	26	19	16	14	$T = 157.972 - 8.939t + 0.139t^2$
	(26-27)	(19-21)	(15-16)	(13-15)	(r = 0.997)
Hatch out	32	24	20	17	$T = 171.639 - 9.272t + 0.139t^2$
	(31-35)	(23-26)	(19-21)	(16-18)	(r = 0.998)

*Range of time is shown in parenthesis under mean time.
**Equation between the time and temperature, $T = a + bt + ct^2$ (T = Time, h; t = temperature, °C).

INCUBATION PERIOD

No eggs hatched out at the incubation temperature of 20°C. Average time to 50% hatching at 23, 26, 29, 32 and 35°C was 32.4, 24.0, 19.5, 16.5 and 15.8 h after spawning, respectively. The relationship between time to 50% hatching (T hours) and incubation temperature (23°C <t< 35°C) can be expressed as follows (Fig. 2):

$$T = 171.371 - 9.136t + 0.134t^2 \ (r = 0.999)$$

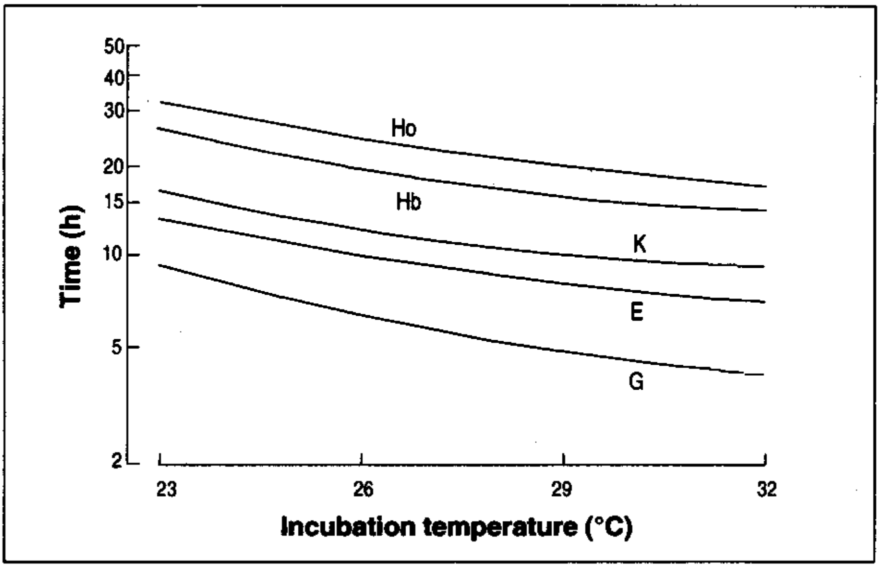

Fig 1. Relationship between incubation temperature and time from spawning to various embryonic stages in *Epinephelus coioides* eggs. G: gastrula stage, E: appearance of embryo, K: appearance of Kupffer's vesicle, Hb: commencement of heart beat, Ho: Hatch out.

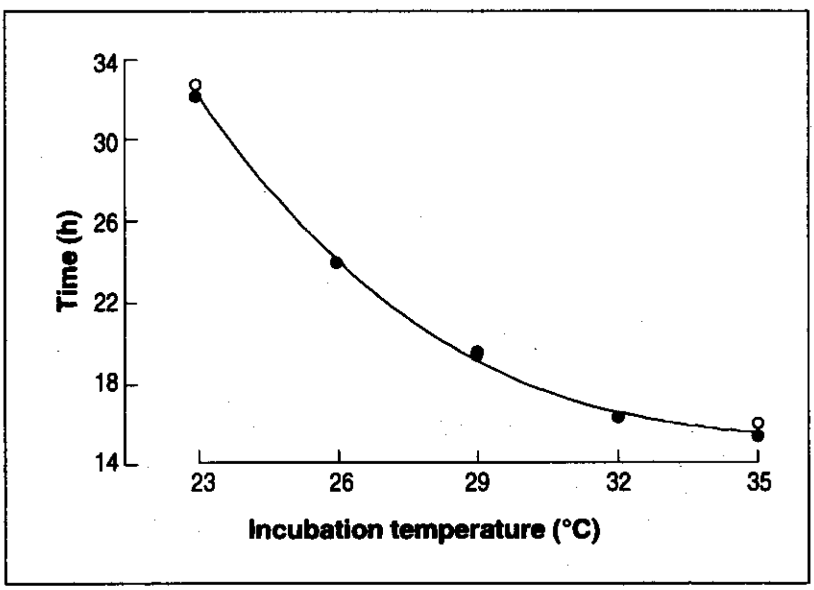

Fig. 2. Time from spawning to hatching under various incubation temperatures in *Epinephelus coioides* eggs. Eggs spawned on 1 March (O) and 4 March (●).

HATCHABILITY

Total hatching rates (TH) and viable hatching rates (VH) of fertilized eggs spawned at 23, 25 and 27°C, and incubated at 20-34°C are shown in Table 2. Hatching occurred at incubation temperatures of 22-34°C regardless of spawning temperature. High TH of more than 80% was recorded with eggs spawned at 23°C and incubated at 22-28°C, and with eggs spawned at 25 and 27°C and incubated at 24-30°C (Fig. 3A). Consistently high VH of more than 80% was attained at incubation temperatures of 24-28°C (Fig. 3B). At incubation temperatures of 22, 32 and 34°C, much higher rates of abnormal larvae were obtained than at 24-28°C (Fig. 3C). Lower and upper limiting incubation temperatures (LLT and ULT) for 50% TH and 50% VH were estimated by linear interpolation following Kashiwagi et al. (1990) (Fig. 4). Average LLT and ULT for 50% TH increased 1.8°C (21.3-23.1°C) and 1.2°C (31.6-32.8°C), respectively, with 4°C increase of spawning temperature. Changes of both LLT and ULT for 50% VH (22.1-23.2°C and 30.1-31.0°C) were small between spawning temperatures.

Table 2. The percentages of total hatching (TH) and viable hatching (VH) of fertilized *Epinephelus coioides* eggs spawned at different water temperatures under various incubation temperatures[1].

Parameters	Temperature at spawning (°C)	Spawning date (in 1993)	Incubation temperature[2] (°C)						
			22	24	26	28	30	32	34
TH	23	16/2	65.2	96.2	86.4	90.2	67.4	45.7	4.3
		21/2	93.2	93.5	89.8	85.1	67.3	46.2	2.2
	25	18/3	26.3	94.5	91.1	92.7	92.7	62.4	1.2
		22/3	9.8	87.9	94.4	98.2	90.2	71.8	2.6
	27	15/4	4.2	91.2	98.3	92.9	93.8	84.8	7.5
		20/4	2.1	87.2	96.3	89.6	77.9	66.6	9.6
VH	23	16/2	34.8	95.0	84.8	87.6	53.3	11.7	0.0
		21/2	61.7	92.1	88.4	83.9	51.8	9.2	0.0
	25	18/3	13.0	91.9	86.1	89.9	78.0	11.5	0.0
		22/3	5.1	84.4	88.8	94.2	75.8	18.2	0.0
	27	15/4	1.5	87.9	94.0	89.2	88.8	22.7	0.0
		20/4	0.5	83.2	87.7	82.1	70.4	16.8	0.0

[1] Each value is the mean of duplicate.
[2] The data at 20°C are omitted because no eggs hatched.

Salinity Experiment

INCUBATION PERIOD

Results of the incubation period test are shown in Fig. 5. No eggs hatched out at 0 ppt. At an incubation temperature of 25.2°C, time to 50% hatching at salinities of 7-57 ppt was 25.7-27.0 h after spawning. At an incubation temperature of 27.4°C, it was 20.5-21.8 h. For both incubation temperatures, the shortest times were recorded at 37 ppt. No significant difference, however, was noted in hatching times between 17, 27, 37 and 47 ppt (Kolmogorov-Smirnov Two Sample Test, P>0.05).

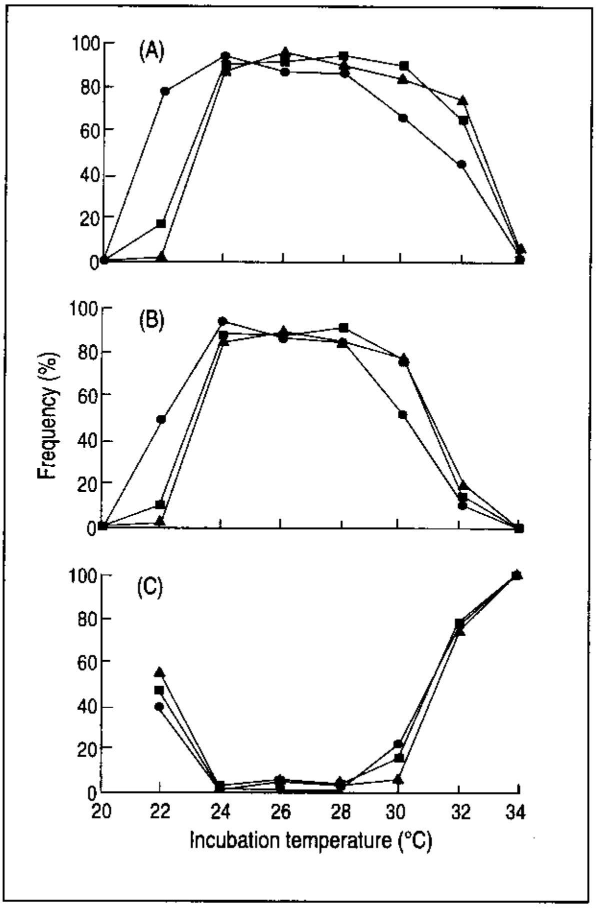

Fig. 3. Comparison of total hatching rates (A), viable hatching rates (B) and abnormal larvae rates (C) of fertilized *Epinephelus coioides* eggs under various incubation temperatures among different spawning temperatures (●--● 23°C, ■—■ 25°C, and ▲--▲ 27°C). Each dot indicates a mean value.

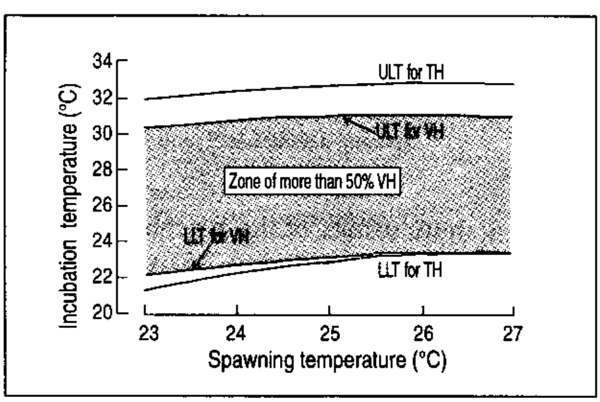

Fig. 4. Changes of lower and upper limiting incubation temperatures (LLT and ULT) for 50% total and 50% viable hatching (50% TH and 50% VH) of fertilized *Epinephelus coioides* eggs with spawning temperature.

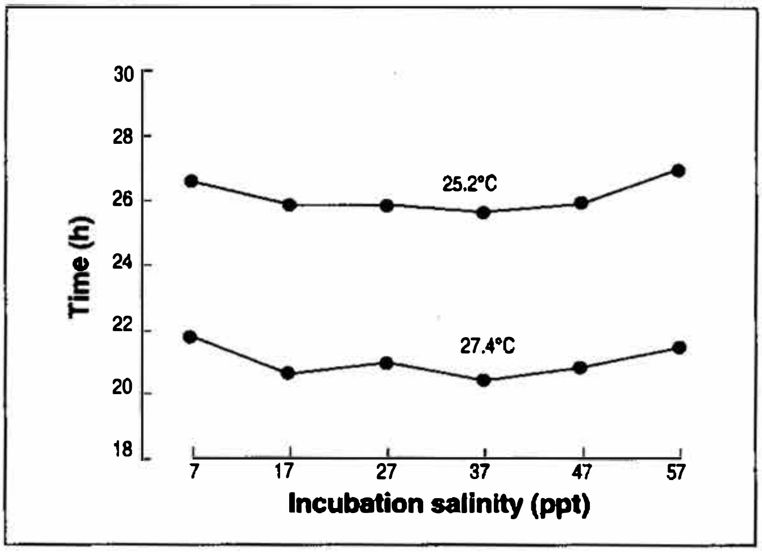

Fig. 5. Time from spawning to hatching under various incubation salinities at incubation temperatures of 25.2 and 27.4°C in *Epinephelus coioides* eggs.

Table 3. The percentages of total hatching (TH) and viable hatching (VH) of fertilized *Epinephelus coioides* eggs under various incubation salinities[1].

Parameters	Spawning date (in 1993)	Incubation salinity[2] (ppt)					
		7	17	27	37	47	57
TH	21/6	71.1	85.7	82.0	91.9	88.8	72.4
	24/6	81.8	87.0	89.2	84.3	92.4	62.2
VH	21/6	18.5	80.0	80.7	89.3	86.5	8.1
	24/6	11.1	84.4	88.3	84.3	91.9	6.7

[1] Each value is the mean of duplicate.
[2] The data with 0 ppt are omitted because no eggs hatched.

HATCHABILITY

Table 3 and Fig. 6 show results of the hatchability test. No eggs hatched out at 0 ppt salinity. The eggs incubated at 7-57 ppt attained more than 50% TH all the time. However, TH in 57 ppt was significantly lower than those in other incubation salinities ($P<0.05$). High abnormality rates of hatched larvae (more than 80%) were recorded in 7 and 57 ppt. VH at 17-47 ppt were constantly more than 80% and had no significant difference ($P<0.05$).

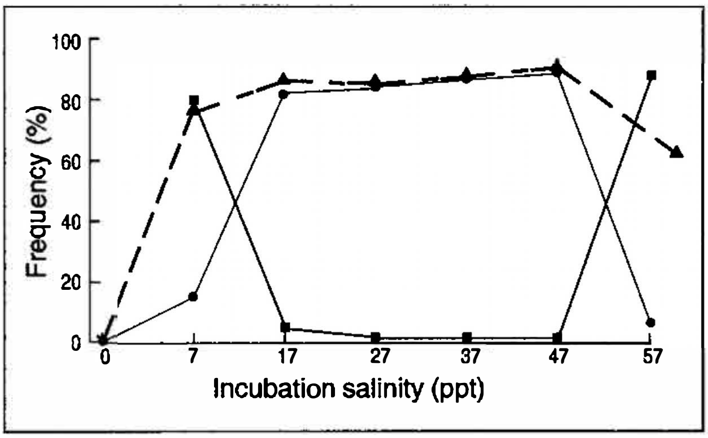

Fig. 6. Total hatching rates (▲--▲), viable hatching rates (●—●) and abnormal larvae rates (■—■) of fertilized *Epinephelus coioides* eggs under various incubation salinities. Each dot indicates a mean value.

Discussion

Time to hatching after fertilization varies, not only with species, but also with incubation temperature (Iwai and Kashiwagi 1989). Hatching time (T hours) is inversely related to temperature (t °C), and it is expressed by the polynomial equation $T = a + bt + ct^2 + dt^3 \ldots$ (Kashiwagi et al. 1986; Kawabe et al. 1991). In the present study, *E. coioides* eggs hatched 32.4, 24.0, 19.5 and 16.5 h after fertilization at 23, 26, 29 and 32°C, respectively. Hussain et al. (1975) reported in Kuwait that this grouper's (referred to as *E. tauvina*) eggs hatched in 26-35 h at 27-30°C, much longer than the present observation. In other *Epinephelus* species, hatching time is comparable to *E. coioides* at specified incubation temperatures (Table 4).

The relationship between time from spawning to each egg developmental stage and the incubation temperature was also expressed by the second order polynomial equation. It appears that egg development for each incubation temperature proceeded at a uniform rate. These findings follow those reported for threeline grunt (*Paraplistipoma trilineatum*) by Kashiwagi et al. (1984) and yellowtail flounder (*Limanda ferruginea*) by Laurence and Howell (1981).

Table 4. Hatching time for various *Epinephelus* species.

Species	Time (h)	Incubation temperature (°C)	Sources
E. fuscoguttatus	18-19	28-30	Chao et al. (1993)
E. malabaricus	19	29-30	Maneewong et al. (1986)
E. microdon	21	26.7-27.7	Tawada (1989)
E. tauvina	23-25	27.0	Chen et al. (1977)
E. akaara	25	25.1	Kayano and Oda (1991)
E. salmonoides	32	24.5	Hamamoto et al. (1986)
E. moara	33	23.5-23.8	Nakagawa (1988)
E. septemfasciatus	30-35	21.2-23.2	Kitajima et al. (1991)

For Japanese whiting (*Sillago japonica*), both the upper and lower limiting incubation temperatures for 50% total hatching and 50% viable hatching rose more than 3°C when spawning temperature increased from 24 to 29°C (Kashiwagi et al. 1987). A similar observation is obtained in *E. coioides*. However, the increase in upper and lower limiting incubation temperatures is much smaller than in Japanese whiting.

Rates of abnormal newly-hatched larvae suggest that the optimum incubation temperature for *E. coioides* eggs is 24-28°C. In Kuwait, this species spawned in the warmer months of April-July when water temperature increases from 23 to 27°C (species referred to as *E. tauvina* in Hussain and Abdullah 1980). This correlates well with the results of the present study. Natural spawning of this species in tanks with 28-32°C water was reported in Malaysia (species referred to as *E. suillus* in Doi et al. 1991). However, the condition of eggs and larvae was not monitored by Doi et al (1991). Iwai and Kashiwagi (1989) summarized the limiting incubation temperatures and salinities for 50% viable hatching of 12 fish species. The largest range of hatchability (18.0°C) was reported for ayu (*Plecoglossus altivelis*). The smallest range (2.2°C) was reported for Petrale sole (*Eopsetta jordani*). With an 8.0°C range, *E. coioides* eggs are considered to have a moderate thermal hatching range. No information on the hatchable range in other *Epinephelus* species is available.

Incubation salinity (7-57 ppt) had little impact on time to hatching in comparison with incubation temperature in *E. coioides* eggs. The same effect has been reported in several other fishes, such as Atlantic herring (*Clupea harengus*) (Blaxter 1969), the threeline grunt (Kashiwagi et al. 1984) and the ayu (Kashiwagi et al. 1986). Incubation salinity of <7 ppt and >57 ppt resulted in longer hatching times. This may be related to high numbers of abnormal larvae at these salinity extremes.

There was no significant difference in VH rate at incubation salinities of 17-47 ppt. This range is considerably wider than the 7.5 ppt range (30.6-38.1 ppt) in threeline grunt, and the 13.8 ppt range (21.8-35.6 ppt) in Petrale sole; and it is similar to that in Pacific cod, *Clupea pallasi* (5-41.9 ppt); horse mackerel, *Truchurus japonicus* (14-40 ppt); red sea bream, *Pagrus major* (11-40 ppt); knifejaw, *Oplegnathus punctatus* (22 to >50 ppt); and English sole, *Parophrys vetulus* (8.7-41.0 ppt) (Iwai and Kashiwagi 1989). On the basis of this comparison, *E. coioides* eggs appear to be euryhaline. This species is often found in brackish areas and penetrates water of very low salinity in rivers, while it occurs in the Arabian Gulf where salinity is often more than 40 ppt (Randall and Heemstra 1991). Natural spawning of the species had occurred at 28-32 ppt in Malaysia (Doi et al. 1991). Results here are consistent with these reports.

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