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Spawning of the Camouflage Grouper *Epinephelus polyphekadion* (Bleeker) in the Hypersaline Waters of Saudi Arabia

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Abstract

Successful natural spawning of the camouflage grouper *Epinephelus polyphekadion* (Bleeker) in captivity, during a 1992-95 culture period, under hypersaline water conditions and environment (salinity 43‰ and temperature 29-30°C) of the Red Sea coast in the Middle East is described for the first time. Until the 1994 culture period, the fish spawned naturally once or twice a year during May or June. Continuous natural spawning of the grouper during the 1995 culture period was observed only after incorporation of polyunsaturated fatty acids in the broodstock diet. Each spawning occurred immediately after the new moon phase, exhibiting a typical lunar rhythm.

Egg diameter varied from 710.0±27.8 µm to 788.0±22.4 µm. There was a linear relation between egg size and egg hatching rate. The increase in hatching rate relevant to egg size was statistically significant ($P<0.01$). Compared to previous spawning records, the fertilization and hatching rates increased significantly ($P<0.05$) from the 1992 to the 1995 spawning period. Egg fertilization rate was 90-100% with a mean of 96.5±3.38%. Egg hatching rate was 70-95% with a mean of 83.14±10.12%. Mean egg fertilization as well as the hatching rates observed during this investigation were higher than in previous records obtained during natural spawnings of other grouper species. Egg development lasted for 19 h. Newly hatched larvae were 1.713+0.119 mm in total length carrying a large yolk sac of 766 µm.

Introduction

The epinepheline serranid groupers, locally known in the Kingdom of Saudi Arabia as najil, taradi and kusher, are commercially important, highly priced fish species. Among 25 species of groupers reported from the Red Sea (Heemstra and Randall 1993), the species with commercial importance are *Epinephelus fuscoguttatus* (brown marbled grouper), *E. polyphekadion* (syn. *E. microdon*, camouflage grouper), *E. malabaricus* (Malabar grouper), *E. coioides* (orange-spotted grouper), *E. tauvina* (greasy grouper), *E. summana* (summan grouper), *Plectropomus maculatus* (spotted coral trout), *P. aerolatus* (square tail coral trout) and *Variola louti* (moontail seabass). In the Middle East, Kuwait was the first country to report on the natural spawning of *E. coioides*, previously reported as *E. tauvina*, for aquaculture (Hussain and Higuchi 1980).

The major constraint for largescale commercial farming of groupers is the non-availability and shortage of fingerlings from the wild or from the hatchery. Most growout production systems for groupers depend on seeds collected from the wild (Quinitio and Toledo 1991), which is unreliable due to seasonal variations depending on the species. Controlled breeding and hatchery production of grouper fingerlings for commercial farming is still in its initial stages of development. The hatchery production of grouper fingerlings is constrained by difficulties in broodstock management to achieve natural spawning in captivity and poor larval survival (Tookwinas 1990).

The shortage of fry, the high market value, and scarcity of groupers from the wild have encouraged many countries to initiate R&D programs on grouper breeding and fry production. Research efforts have been focused on identifying the cycle of reproduction and evaluating sex reversal phenomena of this hermaphrodite species (Debas et al. 1989). Although induced and natural spawning of groupers have been reported from several countries (Chao et al. 1993; Ruangpanit et al. 1993; Toledo et al. 1993), there is not much information available on the natural spawning of *E. polyphekadion* (Bleeker) under captive conditions. Furthermore, due to difficulties encountered in broodstock management, several reports discuss the induced spawning of groupers using hormones (Marte 1990; Tucker et al. 1991; Lim 1993; Tucker and Fitzgerald 1994). This is the first report on a successful year-round natural spawning of the grouper *E. polyphekadion* in captivity under hypersaline water conditions and environment of the Red Sea coast in the Middle East.

Materials and Methods

Wild broodstock of *E. polyphekadion* (2.5-5.4 kg individual size, 54-67 cm total length), were collected in 1991-94 from the hypersaline waters (42‰ salinity) of the Red Sea coast near Jeddah, using hook and line and fish traps. The grouper *E. polyphekadion* was isolated from other grouper species collected from the Red Sea, using the FAO species catalogue (Heemstra and Randall 1993), morphological body characters, and following taxonomic keys: lower gill rakers 8-10 on upper limb, 15-17 on lower limb; pectoral fin-rays 16-17 and number of pyloric caeca 11. Though *E. polyphekadion* is often confused with *E. fuscoguttatus*, the above taxonomic features distinguish the species from *E. fuscoguttatus*. *E. polyphekadion* is the valid name of this species that most recent authors have reported as *E. microdon*.

The fish were stocked in two 25-m^3 capacity broodstock holding tanks at a stocking rate of 2-3 kg m^{-3}. The sex ratio was kept at two female per male (2:1). The seawater used in the facility was taken from borewells dug near a coral lagoon. Water salinity was 43‰. The seawater level in each tank was maintained at a depth of 2 m, water flow rate was maintained at about 5 m^3 h^{-1}. Water temperature was maintained at 29-30°C throughout the year. Until the 1994 spawning period, the fish were fed daily with sardines and squid at 1% biomass. From December 1994 onwards, the fish were fed to satiation daily

with fresh sardines enriched with cod liver oil by injecting 1 ml of the commercial product 'Sevenseas' into the gut of each sardine.

Using a glass bottom viewer, the breeders were observed daily for gonadal development, mating activities and spawning. The fish were allowed to spawn naturally. Whenever spawning occurred, the eggs were collected using a 200-μm mesh size plankton scoop net. The eggs were rinsed with clean seawater and transferred to 1,000- and 2,000-ml graduated cylinders to measure the volume of buoyant and sunken eggs. Samples of eggs were also examined under a compound microscope to record the egg diameter, oil globule and fertilization rate. Buoyant eggs were incubated at 29°C in 0.2-1.8 m^3 capacity fiberglass tanks at a stocking density of 300-500 eggs l^{-1}. The incubation tanks were moderately aerated and the water was allowed to flow through to achieve about 300% d^{-1} water exchange. Egg hatching rates were calculated from the incubated buoyant eggs, and expressed as percent of the buoyant eggs only. The embryonic development of the egg was monitored using a compound microscope, and the development time at different stages were recorded.

Results

Until the 1994 culture period, *E. polyphekadion* spawned naturally once or twice in May or June. Spawning occurred in the morning at about 6:00 hours immediately after the new moon, exhibiting a lunar rhythm. During the 1992 culture period, the fish in one of the broodstock tanks spawned naturally on 22-23 May and on 23-25 June yielding about 2.4 x 10^6 eggs. Egg fertilization rate was 60-85% with a mean of 72.45±10.19%. Egg hatching rate was 65-77% with a mean of 73.56±5.84%. In 1993, the fish spawned naturally only once on 18 May in both of the broodstock tanks, yielding about 4.5 x 10^6 eggs. Egg fertilization rate was 82%. Egg hatching rate was 78%. In the 1994 culture period, the fish spawned naturally only once on 11 May in both broodstock holding tanks, yielding 7.0 x 10^6 eggs. Egg fertilization rate was 90% and hatching rate was about 84%.

E. polyphekadion spawned naturally in the broodstock holding tanks in March, April, May and August 1995. Each spawning run lasted for 2-3 d after the new moon. During the initial spawning in March, 1.249 x 10^6 eggs were obtained. The peak period of spawning occurred in April when 2.59 x 10^6 eggs were obtained (Fig. 1). Egg production decreased considerably at the end of the spawning period in August yielding only 0.433 x 10^6 eggs. Egg quality, represented by the percentage of viable eggs (buoyant eggs), also decreased from about 80% in March and April to 30.4% in August at the end of the spawning season (Fig.2). The mean percentage of buoyant eggs obtained during the 1995 spawning period was 64.75%. Compared to previous spawning records, egg fertilization and hatching rates increased significantly ($P<0.05$) from the 1992 to the 1995 spawning period (Fig. 3). Egg fertilization rate was 90-100% with a mean of 96.5±3.38%. Egg hatching rate was 70-95% with a mean of 83.14±10.12%.

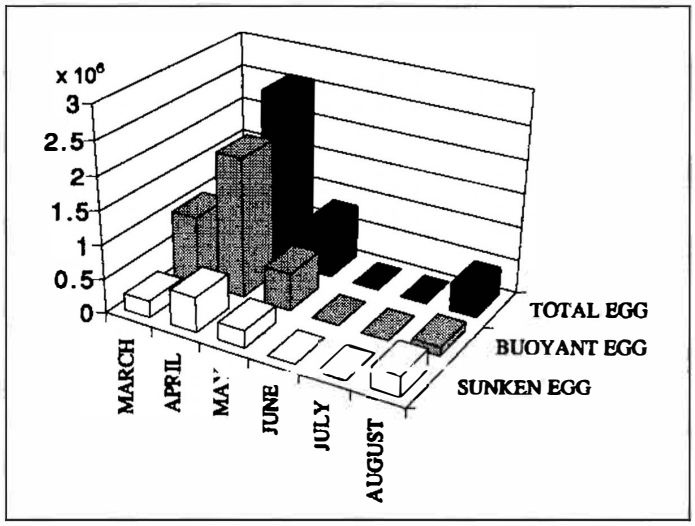


Fig. 1. Spawning period and egg production of *Epinephelus polyphekadion*.

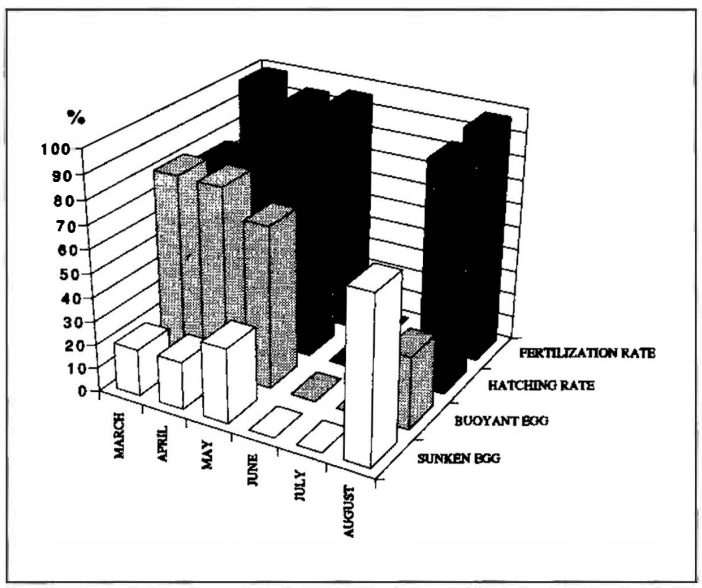


Fig. 2. Egg quality of *Epinephelus polyphekadion* during the spawning season.

Egg diameter varied from 710.0±27.8 μm to 788.0±22.4 μm. Contrary to the decline of buoyant eggs in August (30.4%), egg diameter increased during this period and a maximum of 788-μm diameter eggs were observed in August. The smallest eggs of 710 μm diameter were observed in May after a continuous monthly spawning from March till May. There was a linear relation between egg size and egg hatching rate as represented in Fig. 4 fitted by a linear regression. Dotted lines show the 95% limits of confidence. The increase in the hatching rate relevant to egg size was statistically significant ($P<0.01$).

Figs. 5 and 6 and Table 1 show the egg development of *E. polyphekadion* which lasted for 19 h. The first cell division occurred after 40 minutes of fertilization. Cell division continued every 15-30 minutes until the egg developed into the multicell stage in 2 h and 25 minutes. The egg developed further through the usual stages of blastula, gastrula, embryonic shield, neurula and embryo.

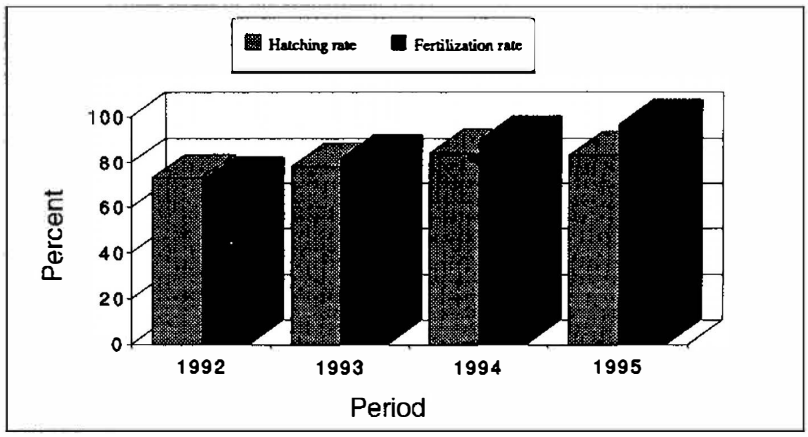


Fig. 3. Egg fertilization and hatching rates of *Epinephelus polyphekadion* from 1992 to 1995 spawning period.

The first movement of the embryo was observed after 16 h. Larvae began to hatch out after 19 h. Newly hatched larvae were 1.713±0.119 mm in total length. Each carried a large yolk sac of 766 µm. The yolk sac had a 181 µm-diameter oil globule which enabled the larvae to be oriented in the water with its head down. The eyes were unpigmented, and the mouth and anus were not open.

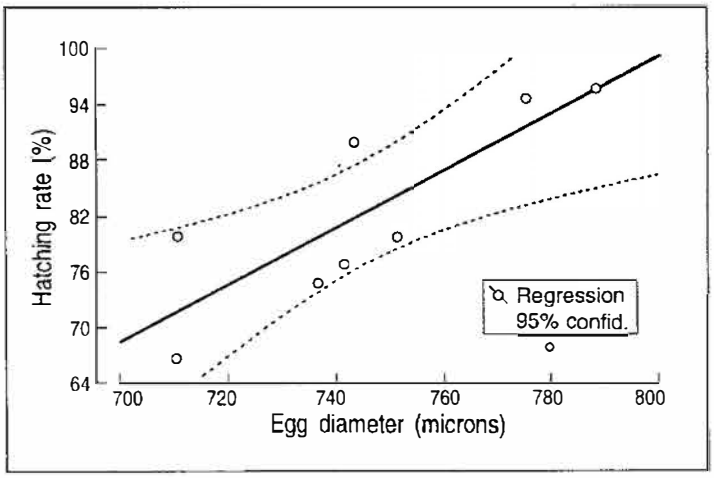


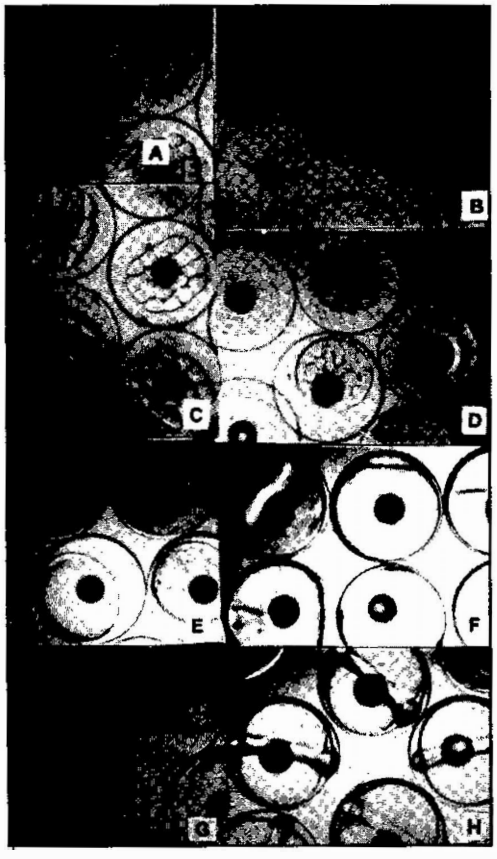
Fig. 4. Linear relation between egg size and egg hatching rate of *Epinephelus polyphekadion*. Dotted lines show the 95% limits of confidence.

Discussion

This is the first report on the spontaneous natural spawning of *E. polyphekadion* for several months, from March to August except June and July, under captive tank culture conditions in the hypersaline waters of the Red Sea coast of Saudi Arabia. The spawning pause observed in June and July could be attributed to the natural ovarian recovery period required by the fish to re-establish their gonads for subsequent spawning as observed in other grouper

Table 1. Stages of egg and embryonic development in the grouper *E. polyphekadion*.

Egg development stage	Time (h)
Fertilized egg	00:00
One cell	00:40
Two cell	01:00
Four cell	01:15
Eight cell	01:30
16 cell	01:45
32 cell	02:00
64 cell	02:25
Morula	02:50
Blastula	03:30
Gastrula	05:45
Embryonic shield covers 1/2 yolk	07:00
Embryonic shield covers 3/4 yolk	08:00
Neurula	08:10
Embryonic body with six myomere	10:30
Embryonic body about 1/2 yolk	11:30
Embryonic body with optic lobe, 16 myomere	12:30
Auditory vesicle appeared	14:30
Optic lens and otolith appeared	14:55
First movement of the embryo	15:55
Heart beat	17:05
Start to hatch out	19:05



species (Lim et al. 1990; Chao et al. 1993; Toledo et al. 1993). While reviewing the environmental influences on gonadal development in fish, Lam (1983) suggested that in tropical and subtropical species, peak spawning activity is often associated with rainfall, floods or the lunar cycle. During the present investigation, *E. polyphekadion* exhibited a typical lunar spawning rhythm, and spawning occurred immediately after the new moon phase. There seems to be no correlation between spawning and rainfall and tidal floods since

Fig. 5. Stages of egg and embryonic development of *Epinephelus polyphekadion*: A. 2-cell stage; B. 4- and 8-cell stage; C. 16-cell stage; D. 32-cell and morula stage; E. Blastula and gastrula stage; F. Neurula stage; G. Development of embryonic shield; H. Embryonic body development (16 myomere, optic lobe).

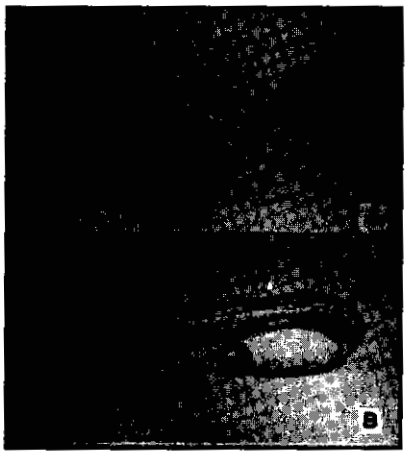


Fig. 6. *Epinephelus polyphekadion* embryonic body development: A. Eggs start to hatch out after 19:05 h incubation; B. Newly hatched larva.

these phenomena are scarce in this part of the Red Sea coast in Saudi Arabia. This is in accordance with the observations of Lim et al. (1990) for *E. fuscoguttatus*. Though spawning occurred immediately after winter, seawater temperature in the broodstock tank was constant year round since there were no severe winter effects at the experimental site. However, the effect of broodstock feed and tank management practices on gonadal development of this species must be further investigated under Saudi environmental conditions, as continuous spawning was obtained during this investigation only after incorporation of poly-unsaturated fatty acids in the broodstock feed. This agrees with the observations of Dhert et al. (1991) who concluded that high levels of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are essential in the broodstock diet to obtain good quality eggs as well as to enhance larval survival in *E. tauvina*. Spawning of groupers occurs in different seasons depending on the species and geographic area (Tucker Jr. et al. 1991; Chao et al. 1993; Ruangpanit et al. 1993; Toledo et al. 1993). Since fully mature *E. polyphekadion* (*E. microdon*) have been caught all year round in Polynesian waters (Debas et al. 1989), and the period of reproduction under culture conditions has been recorded as December-May in the Polynesian waters (AQUACOP et al. 1989), further investigation is necessary to study the possibility of further extending the spawning period of this species under Saudi environmental conditions.

The egg diameter of 710-788 µm observed during this investigation is less than 857 µm recorded for the same species in French Polynesian waters (AQUACOP et al. 1989). Although fertilized eggs of grouper species vary from 700 to 900 µm in size (Fukuhara 1989; Chao et al. 1993), the smaller egg size observed during this investigation will be investigated further to evaluate the effect of broodstock feed and environmental factors on egg size and quality. The smaller egg size observed during this investigation may also be due to the hypersaline water conditions (42-43‰) in the Red Sea since Hussain et al. (1975) observed 770 µm eggs for *E. coioides* (previously recorded as *E. tauvina*) in Kuwait (salinity 42‰), whereas mean egg size of the same species was 840 µm in the less saline waters of the Philippines (Toledo et al. 1993. *E. suillus* is the synonym for *E. coioides*). The increase in mean egg size of 788 µm observed during later spawnings in the present investigation concurs with the observations of Chao et al. (1993) who obtained larger eggs (880 µm) for *E. fuscoguttatus* later in the spawning season. It is interesting to note the significant increase in hatching rate with increase in egg diameter observed in this investigation.

The egg quality represented by the percent of buoyant eggs obtained during this investigation is comparable with previous observations for grouper species. Mean egg fertilization as well as hatching rates in this investigation are higher than previous records obtained during natural spawnings of *E. coioides* (Hussain and Higuchi 1980) in Arabian Gulf waters in Kuwait (9% fertilization and 24% hatching), *E. fuscoguttatus* (Chao et al. 1993) in Singapore (92.2% fertilization), *E. malabaricus* (Ruangpanit et al. 1993) in Thailand (75.7% hatching rate), *E. coioides* (Toledo et al. 1993) in the Philippines (89% fertilization and 81% hatching). The significant increase in egg fertilization and hatching rates obtained in the 1995 spawning period could be related to the enrichment of the broodstock diet with DHA and EPA as observed by Dhert et al. (1991).

The egg development and hatching time of 19 h at a temperature of 29°C is similar to that recorded by Kohno et al. (1990) and Chao et al. (1993) for *E. fuscoguttatus* and Chulavitayanukool et al. (1985) for *E. malabaricus*, and shorter than that of Chen et al. (1977) 23-24 h at 27°C, and 35 h at 28°C for greasy grouper (Hussain and Higuchi 1980). The size of the newly hatched larvae of *E. polyphekadion* (1.713±0.119 mm) observed during this investigation is within the range of 1.8-1.9 mm recorded for *E. fuscoguttatus* (Chao et al. 1993) and 1.45-1.9 mm recorded for red spotted grouper (Fukuhara 1989). However, the newly hatched larval size observed during this investigation is smaller than the 1.92 mm size recorded for *E. malabaricus* (Ruangpanit et al. 1986) and the 2.25 mm larval size observed for the grouper in Kuwait (Hussain and Higuchi 1980).

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