

## Spawning Performance and Larval Rearing of Red Porgy (*Pagrus pagrus* L., 1758) Under Culture Conditions

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**Özet: Fangri balığının (*Pagrus pagrus* L., 1758) yetiştiricilik koşullarındaki yumurta verimi ve larval yetiştiriciliği.** Bu çalışmada fangri balığının (*Pagrus pagrus*) yetiştiricilik ortamında yumurta verimi, larva ve yavru balık üretim potansiyeli incelenmiştir. Anaçlara (1651±38.2 g ortama ağırlığa sahip) 40-50µg/kg miktarında implant tipinde Gn-RHa hormonu uygulanmıştır. Yumurtlama 5 Mart ve 30 Mayıs arasındaki 87 günlük bir dönemde gerçekleşmiştir. Yumurtlama periyodu süresince dişi bireylerin toplam yumurta verimi 550 000 yumurta/kg olarak tespit edilmiştir. Yumurtaların döllenme oranı %80-85 arasında saptanmıştır. Larval dönemde 4-30. günlerde yeşil su tekniği uygulanmıştır. 0-100. günler arasındaki morfolojik gelişme, büyüme ve yaşama oranları belirlenmiştir. Metamorfoz 25-26. günlerde 10,585±0,79 mm total boydaki bireylerde başlayıp 45. günde larvalar 15,33±1,28 mm total boya ulaşmaya kadar sürmüştür. 45. günde 0,049 ± 0,014 g, 100. günde 2,3±0,4g ortalama ağırlığa ulaşmıştır. Yaşama oranları 30. günde %15- %25, 60.günde %12-19 ve 100. günde %8-10 arasında kaydedilmiştir.

**Anahtar Kelimeler:** Fangri, (*Pagrus pagrus*), yumurta verimi, larval gelişim, yaşama oranı, büyüme.

**Abstract:** Egg production, larvae and juvenile rearing potential of red porgy (*Pagrus pagrus*) in captivity were studied through a production season. Female brooders (1651±38.2 g mean body weight) were implanted with silastic capsules carrying GnRH $\alpha$  at a dose of 40-50 µg hormone per kg body weight. Shortly after the GnRH implants, spawning commenced on March 5 and continued up to May 30 over a 87 day period. Through out the spawning season, total fecundity of the females was 550,000 eggs / kg female weight and 80-85% of the eggs were buoyant. Green water technique was used from day 4 to 30. Larval rearing morphologic development, growth and survival rates of red porgy were determined up to day 100. The transformation stage began on day 25 and day 26 at a mean total length of 10.285 ± 0.79 mm and ended on day 45 at a mean total length of 15.33 ± 1.28 mm. Larvae reached the mean weights of 0.049 ± 0.014 and 2.3 ± 0.4 g on days 45 and 100, respectively. Survival rates were respectively 15-25%, 12-19%, 8-10% on days 30, 60 and 100.

**Key Words:** Red porgy (*Pagrus pagrus*), spawning, larval development, survival rate, growth.

### Introduction

Many studies on the broodstock management, embryologic development, larval rearing and growth of red porgy were carried out through out many research and commercial hatcheries in Greece, Spain, Italy, Portugal, Southern Cyprus and Argentina since 1989 (Kolios *et al.*, 1997; Abellan and Basurco 1999; Bodington, 2000; Fostier *et al.*, 2000; Mihelakakis *et al.*, 2001; Machinandiarena *et al.*, 2003). These studies showed that egg production and larval rearing of red porgy in captivity were possible, they can easily adapt to man made environments, survive at high stocking rates and they show high growth performance (Divanach *et al.*, 1993; Kentouri *et al.*, 1995; Cejas *et al.*, 1999). However there are still some important problems faced in intensive rearing conditions which prevent their wide commercial production. One of these problems is low larval survival rates (Conides *et al.*, 2000) and the other is discoloration of market sized fish (Kentouri *et al.*, 1995; Stephanou *et al.*, 1995; Kolios *et al.*, 1997).

This study aimed to determine the culture performance of red porgy as a new species for mariculture in Turkish Aegean coast.

### Materials and Methods

This study was carried out in the hatchery of Ministry of Agriculture and Rural Affairs Hatchery in Bodrum.

Brood fish of regional (wild) origin was obtained from farms collecting small fishes caught in the same region during 1999-2000, and stocked 3.2 m square cages in Güvercinlik bay. In December 2001, brood fish were transferred and stocked 11m<sup>3</sup> indoor concrete tank with a 4.5 kg/m<sup>3</sup> stocking rate. Sex ratio of the brooders was close to 3/1 (20 female and 7 male fish of 4-5 year-old). The mean weight of female fish was 1651±381.2 g (mean±Sd) and mean total length was 38.5±2.07 cm. Mean weight and length of male brooders was 1939±427g and 43.28± 2.81cm.

The colour of the walls and bottom of spawning tank was black. Tank surface was covered with a net. Unheated and unchilled full strength sea water was used in the tanks. Before the spawning period, water exchange rate of broodstock tanks was kept at 30% /h, and during the spawning period reduced to 15% /h. When the brood fish were in the cages, they were fed wet feed which was prepared by mixing fresh fish (*Engraulis encrasicolus*, *Sardina pilchardus*, *Boops boops*)

and pelleted sea bream feed (Pınar Yem A.S.). During the spawning period, fish were fed to satiation with a special broodstock feed (Inve Lansy Inc.) and frozen shrimps at 1:1 ratio. Lighting of the broodstock tanks was adjusted to the natural photoperiod (11-13 h light: 13-11 h dark). Majority of female brooders were implanted with GnRHa silastic capsules carrying 40-50µg hormone / kg body weight (Canario et al., 1997).

The eggs was collected daily and kept in a graduated cyclinder for a 10-15 minutes in order to separate buoyant fertilized from the sinking dead eggs. In the mean time, the total volume of eggs was determined and diameters of 30 eggs were measured. Then, the number of eggs (N) in per ml was calculated with the following equation (Mihelakakis et al., 2001) using the mean diameter (D) of eggs:  $N = 1309 D^{-2.999}$

Eggs were incubated in 100 or 600 l conical tanks after disinfection with 50 ppm iodophore for 10 minutes (Moretti et al., 1999). Stocking rate in incubation tanks was not over 1500 eggs/l. Incubation was carried out in a dark room, with open circulation of sea water of which temperature was 18 °C and salinity was 40 ppt. Water renewal rate was 25% /h. A moderate aeration was provided for homogen distribution of eggs in the tanks.

After hatching, larvae was transferred to the larvae tanks of 8m<sup>3</sup> and 6m<sup>3</sup> at the stocking rates of 75 larvae/litre and 95 larvae/litre, respectively. The walls and bottom of larvae tanks was black. Seawater used in the larvae tanks was filtered through a sand filter (40µm), cardridge filters (25-10 µm), UV disinfected and degassed. Daily water exchange rate was 95% of tank volume between day 0 and day 4 and thereafter, adjusted according to the larval growth rate as shown in Table 1.

Table 1. Daily water renewal rates in larvae tanks (%).

Days posthatch	0-4	4-10	10-15	15-20	25-30	30-	
Water Exchange rate	Daytime	95	-	25	25	50	300-400
	Night		25	25	35-40	70-75	

Larval rearing was carried out in natural sea water temperatures and salinity. Surface skimmers were used from day 4. Photoperiod between day 4 and day 25 was 16 h light: 8 h dark, but it was decreased to 14 h light: 10 hour dark cycle starting on day 25. Illumination was provided by flourescent "cool white" lamps. Light intensity was kept at the level of 300-500 lux at water surface between day 4 and day 25. It was gradually decreased to 70-100 lux by day 25.

Green water technique was used for larval rearing. Feeding was started on day 4. *Nannochloropsis oculata* was supplied into rearing tanks twice a day to achieve and keep a concentration of 1x10<sup>5</sup> cells /ml. During the period between day 25 and day 30, algae concentration in the tanks was decreased from 0.75x10<sup>5</sup> cells/ml to 0 cells/ml. Larvae was fed twice a day with *Brachionus rotundiformis*, which was filtered through 80µm net and kept at a density of 7-10 ind./ml from day 4 to day 13. From day 14 to day 35, *B. plicatilis* at

the same density was used. Rotifers was enriched with an available commercially product (Selco) according to the company's instructions, rinsed and passed through UV disinfection chamber to decrease their surface bacterial flora (Munro et al., 1999). Between the day 22 and day 35, instar I *Artemia* was used at the rate of 0.5-4 million nauplii/m<sup>3</sup>/day. Starting from day 32, instar II *Artemia* was used after enrichment with a commercial product (DHA selco) at a rate of 2-15 million/m<sup>3</sup>/day, but this density later adjusted according to consumption in the tanks. Artificial dry feed was started from day 33 with the 80-100 µm microparticulate feed at a rate of 1-3g/m<sup>3</sup>/day. Inve Inc. dry feeds of 100-200 µm, 300-500 µm, 400-600 µm and 600-800 µm was used starting from days 40, 45, 55, 70 and 85, respectively, according to consumption in the tanks.

Larvae were transferred to adaptation tanks in plastic buckets after decreasing the water in the tanks between day 25 and day 30. Adaptation tanks of 4.5 m<sup>3</sup> and concrete tanks of 8 m<sup>3</sup> were used in the pre-growing phase. Light intensity was lowered to 50-100 lux at this period, since the high light intensity is a reason for juvenile deaths at this stage (Kolios et al., 1997). Seawater filtered with 40 µm sand filter and UV sterilized was used in these tanks. Juveniles were graded at day 45 and day 75 using 4mm grading bars. Before transfer to sea cages, fish without a swimbladder were separated. For the separation process, salinity of the water was raised to 60 ppt and fish anesthetized with 0.3 ppm phenoxy ethanol (Chatain and Corrao, 1992).

Observation on morphology, pigmentation and measurements were made on live larvae. The measurements of larvae were carried out by ocular micrometer under stereo microscope. Dissolved oxygen, temperature and salinity was measured twice a day.

## Results

Spawning commenced on March 5, 2002 and continued up to May 30, over a 87 day period. Total number of eggs spawned in the whole spawning period was approximately 18.2 million. The number of eggs per kg female fish was 550 000. The percentage of fertilized buoyant eggs was 80-85%. Salinity of seawater was 40 ppt. Temperatures during spawning period ranged between 15.8-22 °C. The highest daily egg production was recorded at the water temperatures in between 16.9 °C and 18 °C, between the 3th week of March and the last week of April (Figure 1).

Mean diameter of eggs and oil globule was 990±13µm (mean±Sd) and oil 213±12.9µm. Hatching of eggs occurred within 50-52 h at 18°C with a mean hatching rate of 90-95%. Seawater temperatures in larval rearing periods of 0-10, 10-20, 20-30 and 30-40 days ranged between 18-18.5 °C, 18.5-20 °C, 20-21.5 °C and 21.5-23 °C, respectively. Salinity was 40ppt. Dissolved oxygen concentrations ranged between 4.2-8.3 ppm, but it was generally in between 6 and 7ppm.

Mouth opening in the majority of larvae took place on day 3. By this time, lenght of the first part of the trunk and

pigmentation in the eyes increased. On day 4, yolk sac was totally absorbed, mouth and anus were open and some of the larvae began feeding. As larvae developed, pigmentation on ventral midline between anus and the end of notochord increased. Small groups of stellate melanophores were observed above the gut, on the head and snout tip. Xantophores were observed intensely on head, especially around eyes, and tail. On day 5, the formation of upper and lower jaws took place and the majority of oil globule was consumed. Starting on day 6, initial inflation in swimbladder was observed. The rate of larvae with inflated swim bladder increased while the oil globules of the larvae were completely absorbed. Majority of the larvae completed initial swimbladder inflation by day 9. Second inflation of swim bladder occurred by day 15 while larvae have still primordial fins. Some caudal fin rays and hypurals appeared in the finfold between day 15 and day 20. Flexion in the notochord was prominent during the caudal fin formation. Occipital spine was clearly noticed. Larvae had 22-25 myomeres (Figure 2).

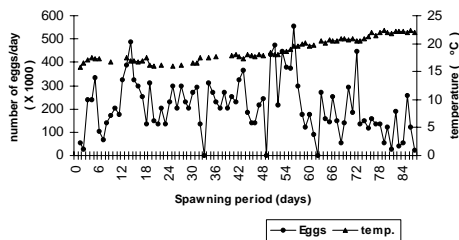


Figure 1. Spawning frequency and number of *P. pagrus* eggs produced daily.

On day 26, the shapes of anal, dorsal, caudal and pectoral fins was prominent. Pigmentation of larvae increased between day 30 and day 37. The head profile was spherical and body depth increased. Pelvic fin rays began to develop. Pigmentation on occipital region increased. Melanophores were present on the maxilleries. A dense pigment patch on lateral surface of caudal peduncle was present. Dorsal, anal, caudal and pelvic fin rays reached to their original number. Original spine numbers (22-23) was observed on day 30. Larvae completed the transformation until day 45. In this period, larval outlook disappears slowly and larvae became opaque. As larvae grow, pigmentation intensified on the trunk. The number of occipital spines decreased. Small melanophores were present on developing dorsal, pelvic and caudal fins. Caudal fin became forked. The development of red porgy between day 25-day 45 was shown in Figure 3.

After day 45, formation of fins was completed and individuals looked similar to the adults. Body surface was covered with ctenoid scales. Pigmentation pattern was uniform and constituted by small stellate melanophores and some vertical lateral bands

Occipital bone completely disappeared. The scales are prominent on day 70 (Figure 4). In this period juveniles have reddish-orange colour.

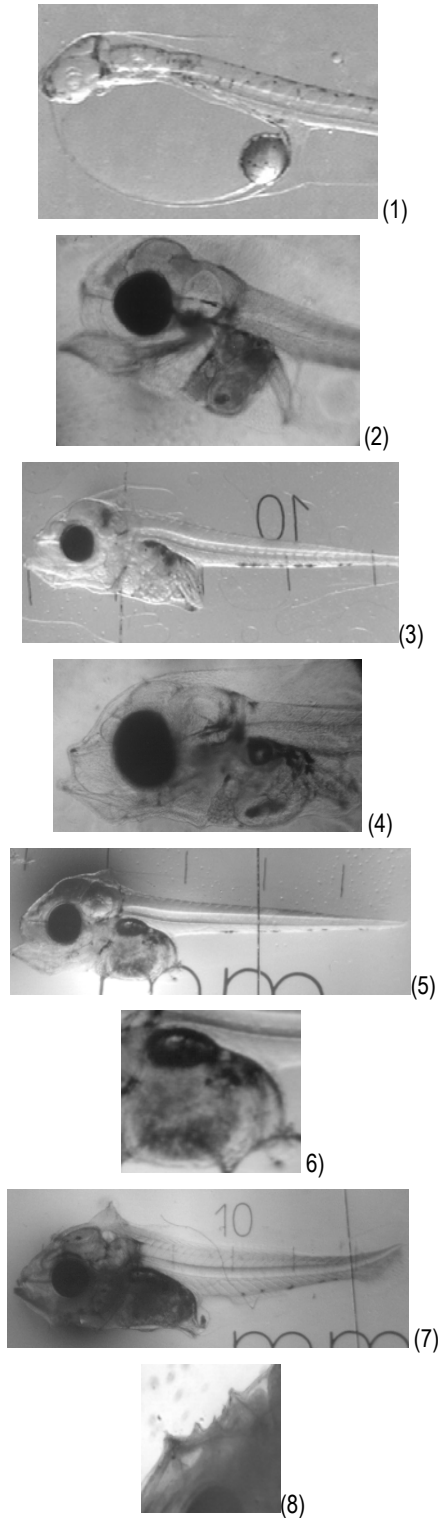


Figure 2. Development of red porgy (*Pagrus pagrus*) between day 4 and day 22 (1) D 0; (2) D5 head and digestive sys.; (3) D10; (4) digestive sys. and swimbladder; (5) D 15; (6) inflated swimbladder; (7) Day 20-22; (8) occipital spine.

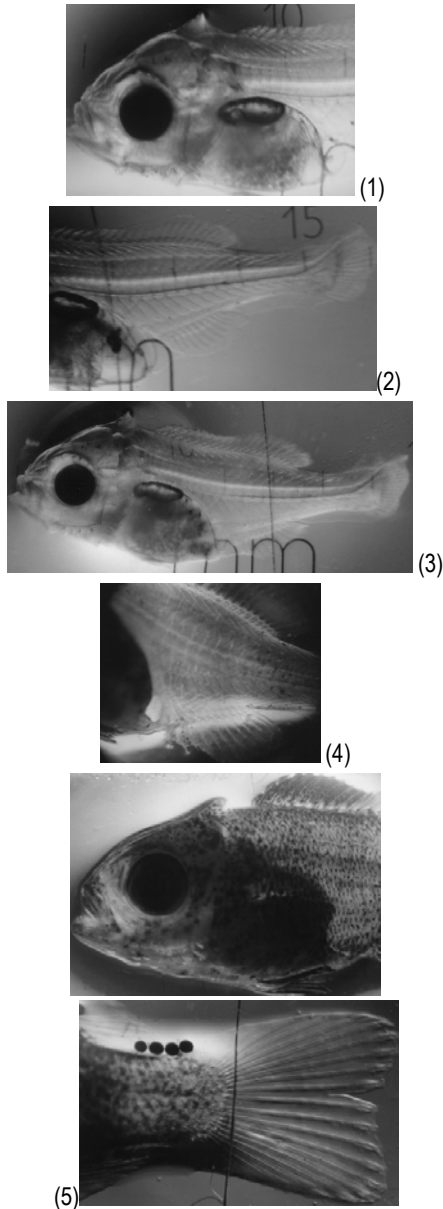


Figure 3. Development of red porgy (*Pagrus pagrus*) between day 25 and day 40 (1) Day 25-26; (2) fin and bone formation; (3) Day 29-30; (4) miomers; (5) Day 40 were seen.

Red porgy larvae reached  $3.95 \pm 0.3$  mm (mean $\pm$ Sd) total length on day 5. Larvae reached  $7.25 \pm 0.94$  mm mean total length and 0.015 g mean weight on day 20 and  $15.33 \pm 1.28$  mm mean total length and  $0.049 \pm 0.014$  g mean weight on day 45. At the end of the nurse period, on day 100, they reached  $48 \pm 11$  mm mean total length and  $2.3 \pm 0.4$  g mean weight (Table 2).

The transformation stage began between day 25 and day 26 at the mean total length of  $10.285 \pm 0.79$  mm and ended on day 45 at mean total length of  $15.33 \pm 1.28$  mm.

Table 2. Growth of red porgy larvae and juveniles.

Days	Mean total length (mm) (mean $\pm$ Sd)	Mean weight (g) (mean $\pm$ Sd)
3-5	$3.95 \pm 0.33$	-
9-10	$4.7 \pm 0.28$	-
19-21	$7.25 \pm 0.94$	0.015
25-27	$10.585 \pm 0.77$	0.028
45-47	$15.33 \pm 1.28$	$0.049 \pm 0.014$
53	$16.63 \pm 2.05$	$0.063 \pm 0.020$
63	$23.6 \pm 2.29$	$0.37 \pm 0.077$
75	$35.1 \pm 3.6$	$0.94 \pm 0.18$
100	$48 \pm 11$	$2.3 \pm 0.4$

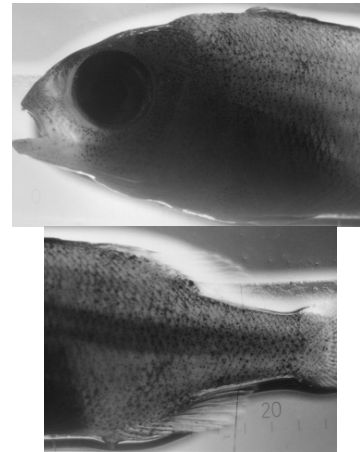


Figure 4. Red porgy juveniles (*Pagrus pagrus*) of day 70.

We recorded two different survival rate in two rearing tank on day 30. The larvae in the tank of  $8 \text{ m}^3$  had a survival of 25% and the larvae in the tank of  $6 \text{ m}^3$  15%. Majority of the mortality (60-75 %) in both tank occurred between day 7 and day 10. Second most important mortality period was between day 22 and day 27 and approximately 10-15 % loss was recorded. Larvae had 12-19 % survival rate on day 60 and survival rate decreased to 8-10 % on day 100. After larvae were transferred to the adaptation tanks, hypertrophy syndrome observed on some of the larvae was the main reason of mortality in this period. Some deaths occurred due to overfeeding with artemia and microparticulate feed, but in between day 35 and day 40 cannibalism was main cause of the deaths (10-15 %). Second important peak in mortality began on day 60 when juveniles reached 0.4 g mean weight and lasted until day 100; approximately half of the juveniles died. The appetite and feeding behaviour of all juveniles before deaths was normal and the stomach of dead ones was full of feed. The percentage of the juveniles without swimbladder separated before transferring to the cages was between 3-7%.

## Discussion

There are some records on spawning of *P. pagrus* in captivity. It was reported that red porgy spawns in the temperature range between 13 and 22 °C which is required for normal embryonic development of this species (Saka et al. 2005). Mendez et al. (1995) reported that broodstock of 3-4 years old

spawned from February 1 to May 13 at the temperature range of 13.6 to 18.3°C. For the same broodstock in the following year Kentouri *et al.* (1995) reported that spawning occurred from February 14 to April 28. Stephanou *et al.* (1995) reported that the spawning of 3-year-old fish occurred from the end of February to end of April within a temperature range from 14.2 °C to 17.8 °C. Mihelakakis *et al.* (2001) reported that spawning of red porgy occurred from February 12 to June 5 in a temperature range from 12.2°C to 18.5°C. In our study spawning occurred in a similar duration with Mihelakakis *et al.* (2001), but spawning continued by the water temperatures of 22 °C. This may have been resulted from the use of GnRHa sustained delivery systems. Zohar *et al.* (1995) reported that the use of hormone implants to induce spawning extended the spawning period up to 4 month. Canario *et al.* (1997) and Zohar and Mylonas (2001) also reported the use of GnRHa in microsphere form increased the spawning period and performance of red porgy. Egg production of red porgy in captivity was reported as 300,000-400,000 eggs/kg body weight by Bodington (2000), 200,000 eggs/kg by Kolios *et al.* (1997), 300,000-500,000 eggs/kg by Stephanou *et al.* (1995), 660,000 eggs/kg by Watanabe and Kiron (1995), 770,000 eggs /Kg by Mihelakakis *et al.* (2001). Our result concerning egg production was different from those of some researchers. These differences may result from factors like the age and the size of brood fish, environmental conditions such as illumination (Kentouri *et al.*, 1995), size and volumes of brood stock tanks (Büke *et al.*, unpublished data) and feeding strategy (Watanabe and Kiron, 1995). Egg diameter of red porgy was determined between 0.64 and 1.09 mm by different researchers, depending on the geographic distribution, feeding strategy, spawning time, spawning method as well as fish age and size (Özden *et al.*, 2005).

Some physical parameters like the temperature result in different developmental sequences of morphological characters (Koumoundouros *et al.*, 2001). The timing of mouth opening, completion of yolk-sac and oil globule absorption in our study showed some small differences with Mihelakakis *et al.* (2001) and Machinandiarena *et al.* (2003). Initial swimbladder inflation in most of the cultured sparidae species occur from day 5 to 7 (Özden *et al.*, 2005). In our study initial swimbladder inflation commenced between days 6 and 9. Findings of Mihelakakis *et al.* (2001) is parallel to ours, but Machinandiarena *et al.* (2003) reported that it lasted by day 12. The size at which notochord flexion begins in sparids is species specific and depends on the size of newly hatched larvae and rearing conditions (Mihelakakis *et al.*, 2001). The formation of notochord flexion was evident from day 20 in our study. Transformation to juvenile stage is under the effect of some environmental factors and temperature is the most important parameter for metamorphosis (Laurance, 1975; Policansky, 1982; Chambers and Legged, 1992). Thus, there are some different records concerning the time of it. Transformation period of red porgy was determined between day 23 and day 32 (at 8.57 - 10.28 mm total length) by Mihelakakis *et al.* (2001) and between day 40 and 51 (at 9.5-

13 mm standart length) by Machinandiarena *et al.* (2003). In our rearing conditions it occurred between day 25 and day 45 (at 10.5-15.3 mm total length).

In different studies, growth as total length was similar in first month. It was 7-7.5 mm on day 20 and 11-12 mm on day 20 and 11-12 mm on day 30 (Kentouri *et al.*, 1995; Roo *et al.*, 1999; Mihelakakis *et al.*, 2001). Mean total length of red porgy juveniles was 40 mm on day 50 (Kentouri *et al.*, 1995) and 4.8 cm on day 90 (Stephanou *et al.*, 1995). Mean weight was reported as 2.98 mg on day 20 (Papandroulakis *et al.*, 2004), 1.4 g on day 80 (Kolios *et al.*, 1997) and 2.3 g on day 90 (Stephanou *et al.*, 1995). When our results were compared with the above studies, growth rates in larval period were higher than the others, but lower in juvenile stage. It may be thought that these differences resulted from different survival rates and feeding strategy in larval period and from digestive problems caused by the feed used in nursery period, which decreased the growth rate of juveniles.

On day 20 of the different studies, carried out by green water rearing techniques, different survival rates of red porgy like 15 % (Hernandez-Cruz, 1999), 40% (Pavlidis *et al.*, 2001) and 48 % (Papandroulakis *et al.*, 2004) were reported. Pavlidis *et al.* (2001) also reported that a survival rate of 50-60 % by semi-extensive rearing method was recorded on day 30. In our study, on day 30 we obtained different survival rates than findings of these researchers. Majority of the deaths (60-75%) occurred between day 7 and day 10. Conides *et al.* (2000) reported similar results. The main reason of deaths in this period was starvation. It may be thought that this situation was related to the fact that larvae could not adapt and digest rotifer. Live feed quality and quantity in the days between 4 and 9 is very important for survival rate (Papandroulakis *et al.*, 2001). Even if the optimum feeding strategy is applied, larvae need a period of several days for learning the hunting behaviour. The survivals of sea bream larvae on day 6 have close relation with the survival on day 15 (Para and Yufera, 2000). Kentouri *et al.* (1995) reported that the mechanic stress caused by the surface cleaners was an important reason of deaths occurred in the early larval period. Another important problem faced in red porgy larval period was related with the behavioural characteristics of larvae with live feed and nutritional quality of feed (Pavlidis *et al.*, 2001). Stephanou *et al.* (1995) and Kentouri *et al.* (1995) reported that transition from rotifer to artemia and increased artemia feeding caused some digestive problems. In our study between day 22 and day 27, we recorded some deaths (10-15%) resulted by the same reasons. Concerning the juvenile stage of different rearing studies, the survival rates of 1-6 % on day 50 (Kentouri *et al.*, 1995) and 5-10 % on day 80 (Kolios *et al.*, 1997) were reported. We recorded the survival rates of 12-19 % on day 60 and 8-10 % on day 100. Kolios *et al.* (1997) reported that increasing light intensity after ending the alg use in the larval tanks was an important factor for deaths in this period. Stephanou *et al.* (1995) reported that losses resulted from digestive disorders after beginning of artificial feed utilization. We observed that, after larvae was transferred to

adaptation tanks, hipertrophy syndrome resulted from stress caused some deaths. The most important losses which occurred in the nursery stage commenced after the increased artificial feed use from day 50. Since the appetite and feed intake of the larvae was normal, it may be thought that this problem results from the fact that digestibility and nutritional properties of microparticulate and granule feeds was not suitable for red porgy. Stephanou et al. (1995) reported similar digestive disorders causing deaths in the same period in where artificial feeding was started.

On day 100, the juveniles without swimbladder was seperated and any other deformity type not observed, however several deformity symptoms which was observed on approximately 30 % of the same fish population became visible later at grow-out stage (Büke et al., unpublished data). It may be attributed to physical rearing conditions and nutritional deficiencies offered by the rearing technique applied during seed production. Divanach and Kentouri (2000) pointed out that larvae in extensive system express important biological rhythms. Patchiness and differential repartition correlated with light, currents or zooplankton concentrations, as well as daily vertical and horizontal migrations are observed. As a consequence, the probability of success is much higher than in the intensive rearing system in which larvae are forced to adapt stringly to environmental and feeding conditions. Koumoundouros et al. (1995) and Divanach et al. (1996) also pointed out that rearing technique affects the larvae quality, and the problems of swimbladder inflation, skeletal deformities, anomalies of coloration and behaviour are almost unknown in extensive techniques.

This study show that the use of GnRH $\alpha$  implants to induce the spawning of breeders in captivity is an effective way of egg production, in case the spontaneous spawning doesn't take place. Further studies should be concentrate on out-of season egg production. Since the mortalities occurred at early larval and juvenile stages coinciding with the start of feeding or introducing a new feed (especially instar II artemia and artificial nurseys feeds), physiological changes and nutritional demand of fish at that age should be examined. Moreover, physical rearing parameters of intensive rearing system need further studies, since they may be reasons of some deformities and low survival rates.

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