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SPAWNING AND LARVAE PRODUCTION OF COMMON PANDORA, *PAGELLUS ERYTHRINUS* L.

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Abstract

Spawning of the common pandora (*Pagellus erythrinus* L.) in captivity was studied at ambient temperature ($21.2 \pm 1.2^\circ\text{C}$) during the breeding season in 1998. Broodstock were cultivated in net fishpens and spawned in large fiberglass tanks (10 m diameter) between June and July. Approximately 33.2 million eggs were collected from 30 mature 4-7 year old females, 97% of which were buoyant healthy eggs with diameters ranging 753-801 μm . The eggs were incubated at 19, 21, or 23°C and development and morphological changes were observed until day 50. The best hatching rate (88.9%) was obtained at 19°C . Swim bladder inflation occurred 8-20 days after hatching. The average survival at day 50, at an initial density of 40 larvae/l, was 3.2%. Exogenous feeding began on day 3. Larvae were first fed rotifers, *Brachionus plicatilis*, and later *Artemia* nauplii. Larvae completed yolk absorption 78 hours after hatching. A green water environment was maintained by introducing cultured microalgae (*Nannochloropsis oculata* and *Tetraselmis suecica*). Larvae (13.3 mm) transformed into juveniles (18.71 mm) 29-37 days after hatching.

Introduction

The Sparidae family has several species that are potentially adaptable to aquaculture (Papastis et al., 1999). Sparidae species are important to marine aquaculture in Mediterranean countries, and numerous investigations have aimed at improving their

artificial reproduction and culture. *Pagellus erythrinus* is a commercially valuable fish in Turkey and other countries along the Mediterranean and Atlantic coasts. Because the demand for *P. erythrinus* exceeds supply, it is being considered as a potential candidate

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for the Mediterranean aquaculture industry (Mihelakakis et al., 2001a).

Information on the biology and reproduction of *P. erythrinus* in nature was reported by Pajuelo and Lorenzo (1998), Benli et al. (2001), and Gonçalves et al. (1997). Fernandez-Pato et al. (1990) and Peleteiro et al. (1997) obtained eggs from *P. bogaraveo* in captivity. Rearing techniques for the mass production of closely related sparids, such as *Sparus aurata* (Polo et al., 1991) and *P. major* (Ikenoue and Kafuku, 1992), already exist. Other species in this family have been studied by Glamuzina et al. (1989), Stephanou et al. (1995), and Mihelakakis et al. (2001b). Cejas et al. (1993) reported preliminary studies on the reproduction and larval rearing of *P. erythrinus* in captivity. Experimental studies on spawning of *P. erythrinus* are in progress at a private fish farm and a research institute in Bodrum, Turkey.

Water parameters directly affect the quality of eggs and larvae. Since *P. erythrinus* has a similar latitudinal distribution, their environmental requirements would be expected to be similar to those of other sparidae. Thus, basics applied to other sparids were tested. Temperature is a major factor that affects egg development in fish (Hansen and Falk-Petersen, 2001). Temperature during incubation may determine morphological traits, hatching rate, behavior, and growth rates of larvae (Hansen and Falk-Petersen, 2001).

The aims of this study were to apply known methods of handling and holding mature sparid broodstock to *P. erythrinus* and determine the fertilization, hatching, and survival rates of *P. erythrinus* eggs and the development and survival rate of larvae in captivity.

Materials and Methods

Broodstock and spawning. The experiment was carried out at the Aquaculture Department of the Faculty of Fisheries at Ege University, Izmir, Turkey. *P. erythrinus* brooders were collected from local estuaries and held in 125 m³ net pens during 1998. They were fed sardines (*Sardina pilchardus*) and 45% protein pelleted feed. After two years of domestication and 2-3 weeks prior to the

spawning season, the brooders were transported from the pens to the hatchery. Thirty selected females measured 28.5-30 cm and weighed 325-377 g. Twelve running males were selected by stripping and measured 26-29.5 cm and 285-370 g.

The brooders were stocked at a male:female ratio of 1:2 at a density of 0.6 kg/m³ in two 14-m³ circular fiberglass tanks with a daily seawater exchange of about 50%. The photoperiod was adjusted to the natural spawning pattern. The water temperature was initially 19.1°C but it was gradually raised to 23.0°C. The mean temperature was 21.2±1.2°C. pH values ranged 6.5-8 and the mean salinity was about 36 ppt. From late April until July, the brooders were fed commercial sea bream pelleted feed (45% protein) daily. During spawning, the brooders were fed fresh or frozen cuttlefish, squid, and mussels.

Eggs were collected daily by the overflow method (Ounais-Guschemann, 1989) in a 500 µm mesh collector. The rate of good quality eggs was determined by dividing the number of floating (good quality) eggs by the number of sinking eggs (poor quality) in a 1-l calibrating cylinder (Carrillo et al., 1989).

Incubation. Batches of 17,500 eggs were incubated and hatched in 4-l cylindrical hatching nets (300 µm mesh size). Three replicates were raised at each temperature (19, 21, and 23°C). Oxygen was provided and eggs were suspended by airstones in each net incubator. Fresh seawater was supplied at a rate of 20% daily. The minimum oxygen level was 5-6 mg/l. Abnormalities, mortalities, and hatching rates were recorded. Fertilization and survival rates were determined by observing embryonic development. Dead eggs were removed periodically and the quantities recorded. Newly hatched larvae were transferred from the hatching nets to 2-m³ cylindrical fiberglass tanks.

Larvae rearing. Two days after hatching, the fiberglass tanks were inoculated with microalgae (*Nannochloropsis oculata* and *Tetraselmis suecica*) at a concentration of approximately 5 x 10⁵ cells/ml to maintain green water culture conditions (Fig. 1). Water temperatures varied 21.4-22.6°C; mean salin-

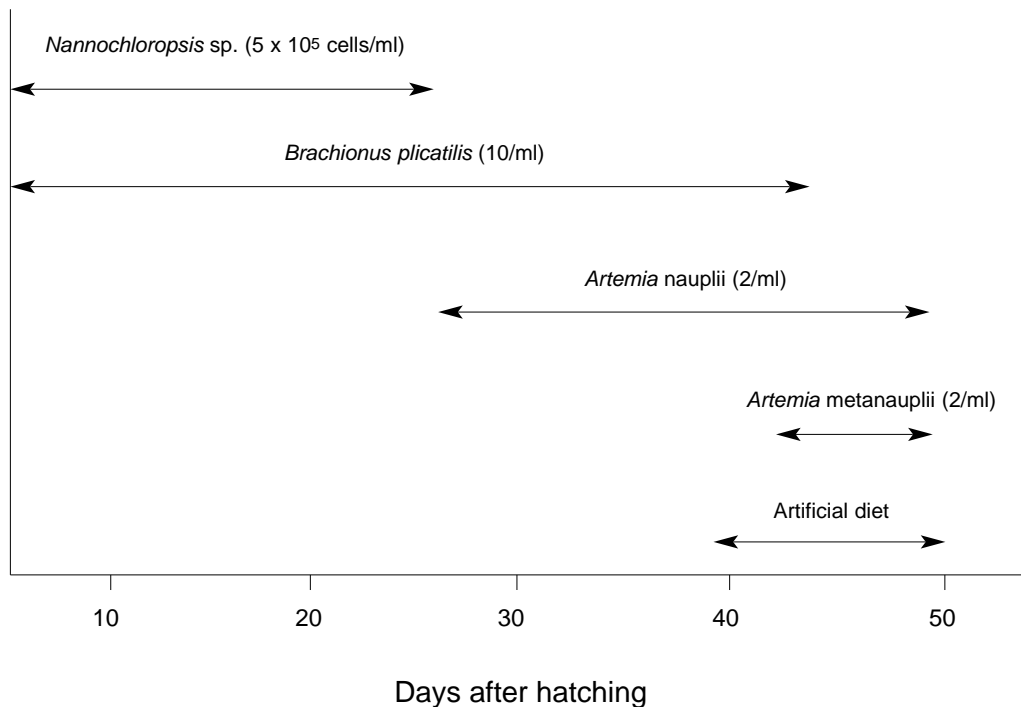


Fig.1. Feeding of *Pagellus erythrinus* larvae.

ity was about 36 ppt. After the mouths of the larvae opened, they were fed rotifers (*Brachionus plicatilis*; L-type), previously enriched with a commercial emulsion (INVE Aquaculture High DHA Selco), at a concentration of 10 rotifers/ml. After the rotifers were added to the tanks, a 50% daily water exchange was maintained.

At 25 days, the larvae reached about 11 ± 0.3 mm. At this stage they were fed *Artemia* nauplii (AF 430 μ m) enriched with commercial emulsion (INVE Aquaculture High DHA Selco) and the daily water exchange rate was doubled (100% per day). The initial ration of *Artemia* nauplii was 2 *Artemia* nauplii/ml. The ration was increased daily together with the water exchange that reached 200% on day 30. A 12h:12h light:dark regime was maintained. Light intensity was measured near the water surface. It was initially 20-50 lux and gradually enhanced to 1000 lux in the

final stage. Wastes in the rearing tank were siphoned out once a day.

Water quality parameters. Water temperature, dissolved oxygen, and pH in the incubators and larvae tanks were monitored three times a day with a digital oxygen meter (WTW model oxygen meter) and pH meter.

Statistical analysis. Experimental data on the duration of embryonic development and hatching rates were analyzed using one-way analysis of variance (ANOVA) to a significance level of $p < 0.05$. Multiple mean comparisons were made with the Tukey test to estimate differences ($p < 0.05$) between results.

Results

Spawning and egg production. Spawning took place during 64 days, from June 5 until August 8, 1998. Forty-two natural spawns were obtained with an average fertilization of $98.8 \pm 1.9\%$ (Fig. 2). The percent of viable

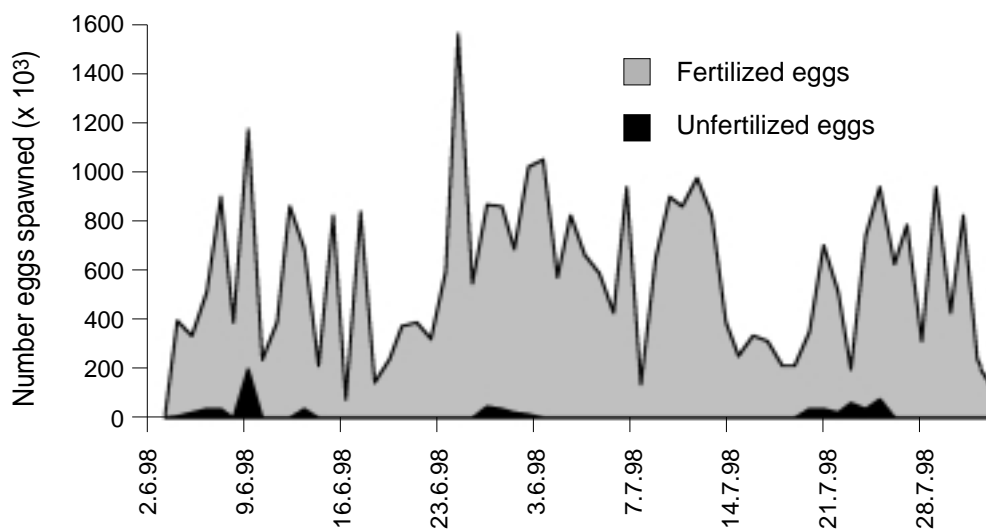


Fig. 2. Number of eggs in broodstock tanks.

eggs was slightly lower, $97 \pm 5.11\%$. The diameter of the viable eggs ranged $753.3\text{--}801.1\text{ }\mu\text{m}$ (mean $777.2 \pm 3.5\text{ }\mu\text{m}$). The females released an average of 3.209 million eggs (8.762 g.) per kg fish.

Hatching rates. Embryonic development varied considerably among the temperature treatments ($p < 0.01$) and decreased nonlinearly as the temperature increased (Table 1). Incubation temperature negatively correlated with hatching time ($r^2 = 0.91$) and percentage ($r^2 = 0.74$, $p < 0.05$) of viable eggs. The percent of abnormal larvae rose with the temperature. Body curvature and tail flexures were the most common abnormalities. There were no significant differences in total length at hatch ($p > 0.05$).

Larvae development. Approximately 250,000 larvae that were hatched at $21 \pm 0.5^\circ\text{C}$ were stocked at an average of 40 larvae/l (Table 2). Skimmers were used to clean the water surface from oily film. Mouth opening occurred 54 h after hatching, before the yolk-sac was absorbed. By 78 hours, when the yolk-sac was completely absorbed, the mouth openings ranged 235–286 μm .

Swim bladder inflation was first observed five days after hatching. The percentage of larvae with an inflated swim bladder was 43% at the end of the experiment. Some larvae reached the juvenile stage (13.34 mm total length) 29 days after hatching, equivalent to 659 degree days (sum of temperatures on each day); 50% reached the juvenile stage 32 days after hatching. By day 37, equivalent to 835 degree days, all were juveniles over 18.71 mm. The mean total length of 50-day old fry was $26.1 \pm 1.2\text{ mm}$ (Fig. 3) and their average weight was $0.41 \pm 0.02\text{ g}$.

The highest mortality (approximately 10%) was recorded during the yolk-sac stage. Dead opaque larvae were removed twice a day. Fifty days after hatching, the survival rates were 3.4%, 4.6% and 1.6%, in tanks 1, 2 and 3, respectively.

Discussion

The common pandora had a prolonged spawning season that lasted from June to August, similar to that reported for the protogynic hermaphrodite pandora in the western Mediterranean (Gonçalves et al., 1997) which

Table 1. Incubation time, hatching rate, total length, yolk diameter, percent anomalies at hatching, and survival of *Pagellus erythrinus* eggs incubated at different temperatures.

| Temperature (°C) | Incubation duration (h) | Hatching rate (%) | Total length at hatching (mm) | Yolk length (mm) | Abnormalities at hatching (%) | Survival* (%) |
|------------------|-------------------------|-------------------|-------------------------------|------------------|-------------------------------|---------------|
| 19 | 70.60±1.09 | 88.9±5.9 | 2.02±0.12 | 0.95±0.03 | 2.68±1.18 | 92.69±1.93 |
| 21 | 54.46±0.55 | 83.4±6.7 | 2.04±0.03 | 0.99±0.04 | 3.65±0.89 | 92.18±2.55 |
| 23 | 51.37±0.60 | 70.4±8.1 | 2.03±0.03 | 0.97±0.03 | 5.35±1.01 | 90.08±3.63 |

* Survival of yolk-sac stage larvae on second day after hatching.

peaks one month earlier than *P. erythrinus*, in May. A spawning period that does not coincide with that of other sparids enables better utilization of sparid hatcheries. The long spawning period indicates nonsynchronous maturation of oocytes. Mature *P. erythrinus* broodstock produced approximately 3.2 million eggs per kg, more than gilthead seabream (*Sparus aurata*), common dentex (*Dentex dentex*), and red seabream (*Pagrus major*; Glamuzina et al., 1989). The estimated fecundity in *P. bogaraveo* ranges 73,000-1.5 million ovocytes for females (29-41 cm total length; Krug, 1990).

The rate of fertilization was very high compared to *D. dentex* (40-80%; Glamuzina et al., 1989), *D. puntazzo* (35-65%), and *D. sargus sargus* (75%; Abellan and Garcia-Alcazar, 1995) but it was similar to other sparids such as *P. pagrus* (95-100%) and *P. major* (96%; Mihelakakis and Yoshimatsu, 1998; Mihelakakis et al., 2001b). The diameter of the *P. erythrinus* eggs was significantly smaller than that of other sparids that range 850-1090 µm (Glamuzina et al., 1989; Greco et al., 1993; Stephanou et al., 1995; Mihelakakis et al., 2001b). Imam and Terzioğlu (2001) found that the mean diameters of eggs and yolk-sac of *P. erythrinus* were 700±10 µm and 100±10 µm, respectively, and that the egg incubation period for *P. erythrinus* was 40 hours at 22°C. *P. bogaraveo* eggs were 1.19±0.0215 mm in diameter, with a single oil drop (0.025 mm diameter), and embryonic development required 58 hours at 14±1°C (Peleteiro et al., 1997).

The results of our study show that temperature affects the incubation period and quality of newly hatched larvae and that the best hatching rate was obtained at 19°C. The time from fertilization to hatching decreased from 70.6 hours at 19°C to 51.37 hours at 23°C, a phenomenon also observed by Pepin (1991) and Mihelakakis and Yoshimatsu (1998). The percentage of abnormalities increased with temperature, a phenomenon also reported by Mihelakakis and Yoshimatsu (1998). Based on the present study, a temperature below 21°C seems most appropriate for optimal hatching, viability, and embryonic development in *P. erythrinus*.

The total length of newly hatched *P. erythrinus* larvae in this study (2.03 mm) was less than in other sparids, e.g., 3.7 mm for *P. bogaraveo* (Peleteiro et al., 1997), 2.2 mm for *P. major* (Lisac, 1989), 2.5-3.2 mm for *P. pagrus* (Stephanou et al., 1995), and 2.3 mm for *D. dentex* larvae (Jug-Dujakovic et al., 1995). This trait may pose some disadvantages for larval feeding and growth. The final total length and growth rates at day 50 for *P. erythrinus* were considerably higher (26.18 mm and 0.42 g) than those obtained for *P. major* (22.8 mm and 0.18 g; Lisac, 1989) and *P. pagrus* (13.5 mm; Mihelakakis et al., 2001b). A better adapted prey size, feeding regime, and rearing temperature may result in higher larvae and juvenile growth rates.

Compared to other cultured marine fish, *P. erythrinus* has a very short yolk-sac stage

Table 2. Larvae development of *Pagellus erythrinus* (means \pm SD; initial stocking rate 40 larvae/l).

| Tank number | Water temperature (°C) | Yolk-sac absorption (h) | Mouth opening | | Swim bladder inflation | | Transformation from larva to juvenile | | Total length at day 50 (mm) | Wet weight at day 50 (g) | Survival at day 50 (%) |
|-------------|------------------------|-------------------------|---------------------|------------------|------------------------|-----------------|---------------------------------------|-------------------|-----------------------------|--------------------------|------------------------|
| | | | Hour after hatching | Size* (μ) | Day of inflation | Rate (%) | Day | Total length (mm) | | | |
| 1 | 21.4 \pm 0.54 | 78.05 \pm 0.59 | 54.19 \pm 0.36 | 255.9 \pm 19.5 | 9-20 | 43.2 \pm 2.05 | 29-35 | 14.4-17.8 | 26.04 \pm 0.63 | 0.42 \pm 0.03 | 3.4 |
| 2 | 22.5 \pm 0.58 | 78.10 \pm 0.56 | 54.23 \pm 0.41 | 264.1 \pm 15.1 | 8-19 | 42.8 \pm 3.48 | 31-36 | 13.5-18.7 | 26.37 \pm 1.34 | 0.41 \pm 0.03 | 4.6 |
| 3 | 22.6 \pm 0.65 | 77.99 \pm 0.31 | 54.16 \pm 0.46 | 261.3 \pm 17.9 | 5-20 | 43.0 \pm 2.39 | 29-37 | 13.3-17.9 | 26.13 \pm 0.84 | 0.42 \pm 0.02 | 1.6 |
| Mean | 22.5 \pm 0.12 | 78.05 \pm 0.49 | 54.19 \pm 0.41 | 260.4 \pm 17.4 | 8-20 | 43.0 \pm 2.64 | 29-37 | 13.3-18.7 | 26.18 \pm 0.94 | 0.42 \pm 0.03 | 3.2 \pm 1.5 |

* 78 h after hatching.

which concurs with the findings of Greco et al. (1993; 3 days at 21.8°C). The mouth opening of *P. erythrinus* was smaller (260.4 μ m) than that of *S. aurata* (437-475 μ m; Glamuzina et al., 1989) and *D. dentex* (256-322 μ m; Jug-Dujakovic et al., 1995). The cultured S-type rotifer is an important initial food for marine fish larvae, particularly those with small mouths (Fukusho and Okauchi, 1983; Polo et al., 1992). The use of S-type rotifers as the initial larvae food enhanced survival rates for a number of marine fish species, such as *S. aurata* in Europe (Fernandez-Diaz et al., 1994) and *P. major* in Japan (Watanabe and Kiron, 1994). However, S-type rotifers (>120 μ m) are not small enough for the initial feeding of *P. erythrinus* larvae. The lack of S-type rotifers *B. plicatilis* may have affected our results.

The high mortality rate (10%) during the early larval stage indicates that modifications are needed in our culture system. Larvae mortality prior to yolk absorption and mouth opening could have been due to starvation or developmental problems related to egg quality. Egg quality is affected by broodstock management (Watanabe et al., 1985; Tandler et al., 1995) and requires further investigation.

The swim bladder inflation rate at day 50 (43%) was less than reported for *S. aurata* (80%; Chatain and Ounais-Guschemann, 1990) and *P. major* (60%; Chatain, 1982). For sparids, a high initial swim bladder inflation rate is beneficial to obtaining good quality fry, as fry without an inflated swim bladder become deformed (Chatain and Dewavrin, 1989).

In conclusion, *P. erythrinus* broodstock can be maintained and spawn naturally in captivity. However, several aspects in the culture of *P. erythrinus* must be improved, mainly handling, holding, maturation of broodstock, and obtaining good quality eggs and larvae. Mortality and morphological deformations, probably caused by unfavorable feeding techniques, require study, together with optimization of the temperature, water exchange rate, illumination, and addition of algae.

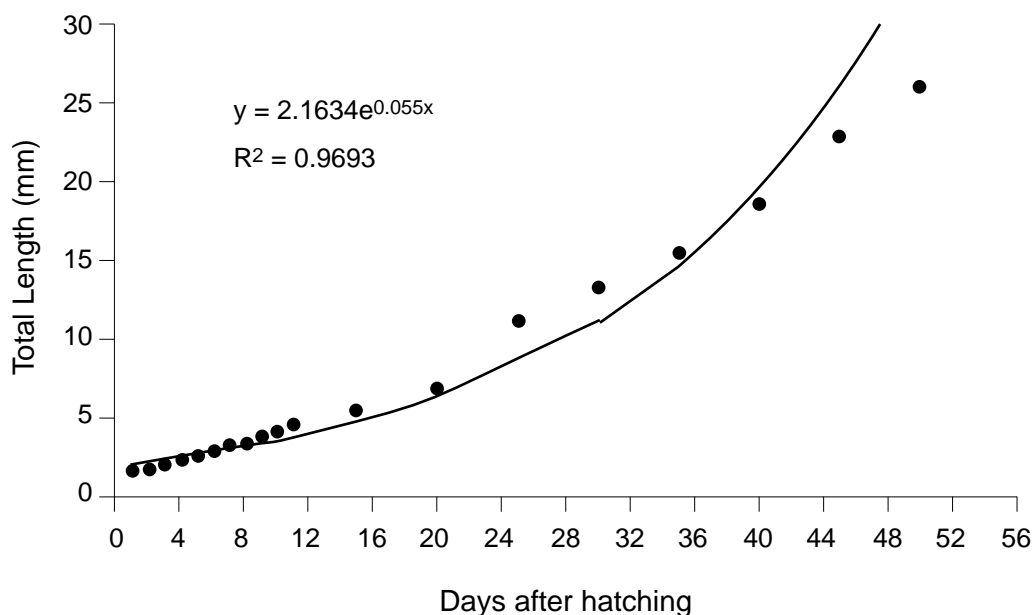


Fig. 3. Growth in *Pagellus erythrinus* larvae.

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