

Full Length Research Paper

# Broodstock feeding effects on spawning performances' (fertility, eggs and larvae quality) of the Mediterranean red porgy (*Pagrus pagrus*, Linnaeus 1758) during two years

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Spawning in captivity of two broodstock groups of red porgy (*Pagrus pagrus*) of the same age was studied during two consecutive spawning seasons. These two broodstock groups, designed as A and B, were fed with different regimes during the spawning period. Group A was fed on moist pellet while group B was fed on fresh fish. Total number of collected eggs was 22 and 36 million respectively for groups A and B during the first year. Fecundity was 474,966 and 769,186 eggs kg<sup>-1</sup> female respectively. During the second year, total number of collected eggs was only 5 million for group A and 32 million for group B. The fecundity was 192,094 and 1.1 million eggs kg<sup>-1</sup> female respectively. Statistically, there was a significant difference between the slopes of the two groups in terms of quantity and quality of spawning and larval survival recorded on the second and fifth day after hatching. The viable egg rate was positively correlated with the second day larval survival, which in turn was significantly correlated with hatching egg rate. These data showed that time of collecting eggs for incubation, does not affect hatching rate and larvae survival rate.

**Key words:** *Pagrus*, red porgy, spawning, egg quality, fertility.

## INTRODUCTION

The red porgy is a common species widely distributed in the Mediterranean Sea and the Atlantic Ocean, with high commercial value (Manoocher and Hassler, 1978; Vassilopoulos and Papaconstantinou, 1992; Harris and McGovern, 1997). The red porgy is a species characterized by a good flesh quality and good market prospects (Basurco and Abellan, 1999). The red porgy fishing is very limited and insufficient comparing with market demand, which makes it as potential species for aquaculture (FAO, 1997).

Regarding its captive culture, the porgy has a high growth rate compared to that of Gilthead sea bream and European sea bass. Data on production, development and nutritional requirements have already been studied

(Kentouri et al., 1994; Kokokiris, 1998; Schuchardt et al., 1999). The red porgy is a hermaphrodite protogyn. Each fish has an ovotestis in which the ovary is the dominant party in the first two years of life (Kokokiris, 1988). The first sexual maturation of red porgy appears at the 3rd year.

Spawning occurs in spring at temperatures of 15 - 19°C. However, specific data on spawning, fertility and eggs quality in captivity are still lacking. The change in the eggs and larvae production was observed in fish farms used to be considered as a limiting factor for the commercial fry production (Kjørsvik et al., 1990). For fish farms, eggs quality can be defined by potential eggs potential to produce viable fry (Kjørsvik et al., 1990). Eggs quality can be influenced by different parameters that often change during the spawning season (quality and food ration for broodstock, physicochemical parameters of breeding water environment, and captive broodstock maintenance (stress, handling, etc.), (Campbell et

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**Table 1.** Composition of the brood stock food.

Ingredients	Sardine <sup>1</sup>	Bogue <sup>2</sup>	Shrimp <sup>3</sup>	Squid <sup>4</sup>	Pellet <sup>5</sup>	Flour <sup>6</sup>	Premix
Group A	50%	0%	0%	0%	45%	3%	2%
Group B	0%	50%	15%	33%	0%	0%	2%

Vitamin premix (dose kg<sup>-1</sup>): Minerals, 75% ; Phosphorous, 3 – 4 % ; Calcium 25 – 30%, Vitamin A, 2.000000 IU ; Vitamin D3, 400000 IU ; Thiamine B1, 500 mg ; Riboflavin B2, 1.000 mg; pantothenate de Calcium B3, 7500 mg; Pyridoxine B6, 500 mg; Vitamin B12, 1,5 mg; Tocophérol Acetate E, 2500 mg; Nicotinic Acid PP, 10000 mg; Choline (Chloride), 50000 mg (Sardina pilchardus),<sup>2</sup> (Boops boops ),<sup>3</sup> (Solenocera membranacea),<sup>4</sup> (Illex coindetii).

al., 1992; Bromage, 1995; Brooks et al., 1997; Christiansen and Torrissen, 1997; Carrillo et al., 2000. Therefore, it is important for all species of aqua-culture interest to conduct research on the existence of egg quality variations, to maximize the eggs and larvae collection in commercial hatcheries. The objective of this work was to contribute to highlight the brood stock feeding effects on captive spawning performances of red porgy in terms of fertility, eggs and larvae quality.

## MATERIALS AND METHODS

### Brood stock

The red porgy broodstock was formed from wild individuals caught at M'diq Bay through small long liners. Caught porgies were kept in polyester rectangular tanks of volume 10m<sup>3</sup>. These parents' fish were fed exclusively fresh fish (sardine) to satisfaction, once a day and six days a week. During the spawning period, they were divided into two groups (A and B). In 2007, group A was composed of two males and forty females and group B was constituted of two males and thirteen females. Both groups were subjected to natural temperature, salinity and photoperiod conditions. The water renewal rate was kept around 300- 500% per day and oxygenation was provided by three air arrivals. The spawning was spontaneous; it took place very early in the morning and fertilization occurred in water. In 2007, the females and males average body weights of both groups A and B were respectively of 3.07 ± 0.5 and 2.89 ± 0.4 kg. The total biomass of females was of 32.50 ± 0.65 kg for group A and 35.58 ± 0.71 kg for group B. Group A received a diet based on moist diet composed of minced sardine 50%, 45% of dry pellet commercial and 3% fish meal flour, complex of vitamin and vitamin C. Group B, was fed by fresh fish with (50%) , Squid (35% )of shrimp (15%) and complex of vitamin and vitamin C were added to the mixture (Table 1). Group A received a diet based on moist diet composed of minced sardine 50%, 45% of dry pellet commercial and 3% fish meal flour, complex of vitamin and vitamin C. Group B, was fed by fresh fish with (50%) , Squid (33%) of shrimp (15%) and complex of vitamin and vitamin C were added to the mixture (Table 1).

In 2008, females number was reduced to 10 in group A (2 fishes lost by death and 2 others changed sex and became males), and to 11 in group B (2 fishes lost by death). The females and males average body weights of both groups A and B were 3.0 ± 0.3kg and 3.4 ± 0.8kg respectively, with a total biomass of females of 47.05 ± 0.75 in group A and 47.66 ± 0.76 kg in group B.

### Evaluation of eggs and larvae quality

During 2007 and 2008 breeding seasons, spawning took place at temperatures ranged between 15.9 and 19.2°C with an average

value of 16.9 ± 0.6°C. The eggs were harvested in collector equipped with a mesh screen net of 500 micrometers. After harvesting, eggs were placed in a small tank of volume 30 L; viable and dead eggs were separated and counted. Eggs and oil globule diameters were measured on a profile projector using digital calliper, through samples taken from each daily collected spawning during 2007 and 2008 breeding seasons. Embryo development and larvae survival were followed using samples of collected eggs which were previously rinsed and stocked in 500ml beakers.

These eggs incubated at room ambient temperature and maintained until the death of all hatched larvae. Embryonic development was evaluated once a day, through profile projector and microscope and microscope observations. The number of hatched eggs and, the number of hatched viable larvae were evaluated daily. Various parameters of eggs quality were evaluated.

The fertilized eggs rate was evaluated every day as soon as the eggs were recuperated from the collector; it was calculated as the eggs number fertilized / total collected. Eggs number. Hatching rate was calculated as the number of hatched larvae / total incubated eggs. Larval survival was estimated as the numbers of live larvae on the second day after hatching (d) / the total hatched larvae number.

### Statistical analysis

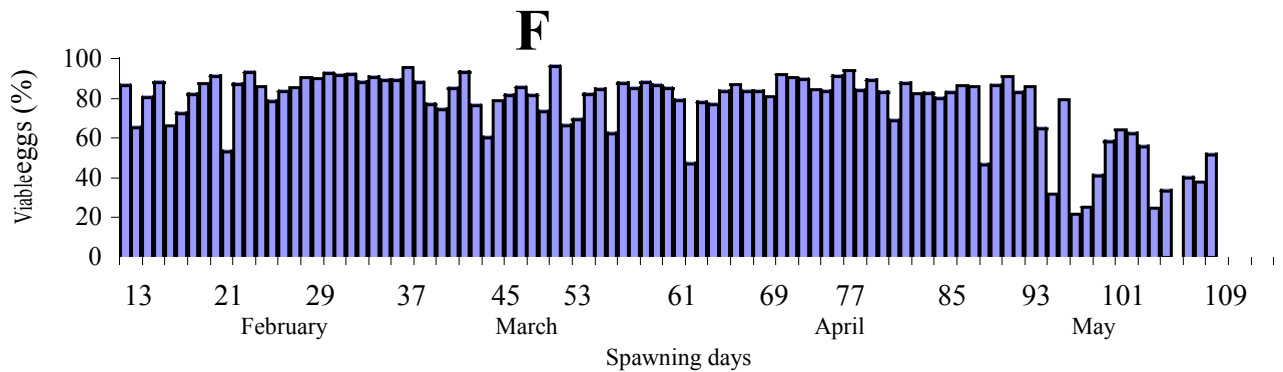
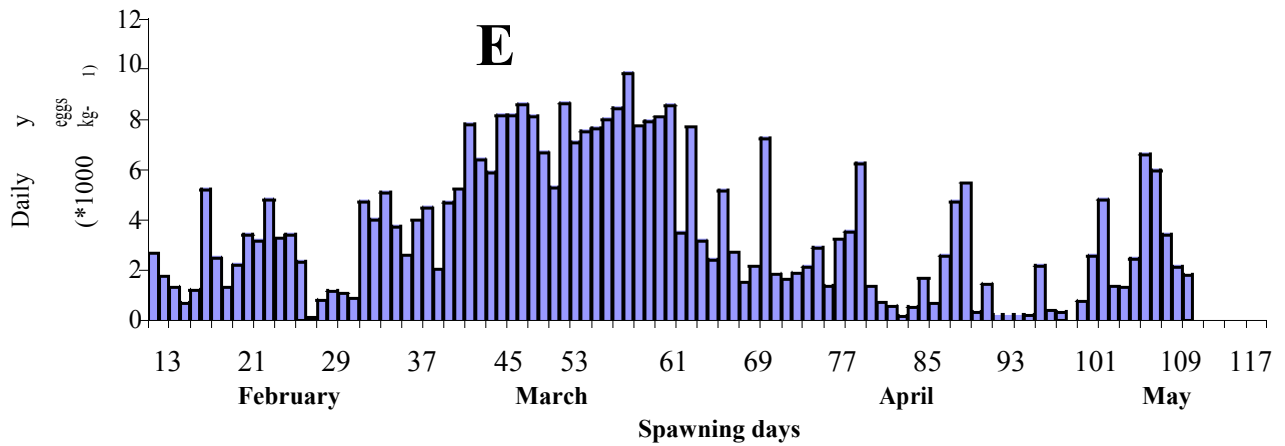
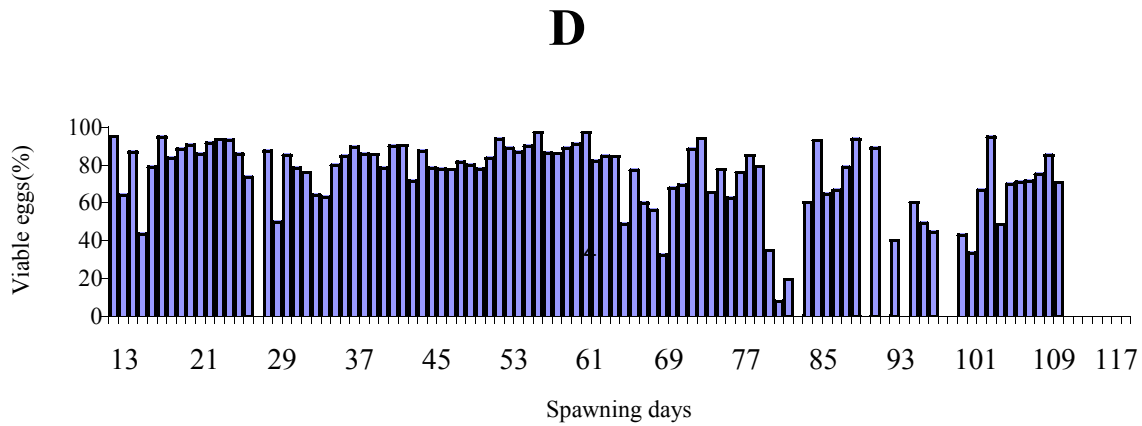
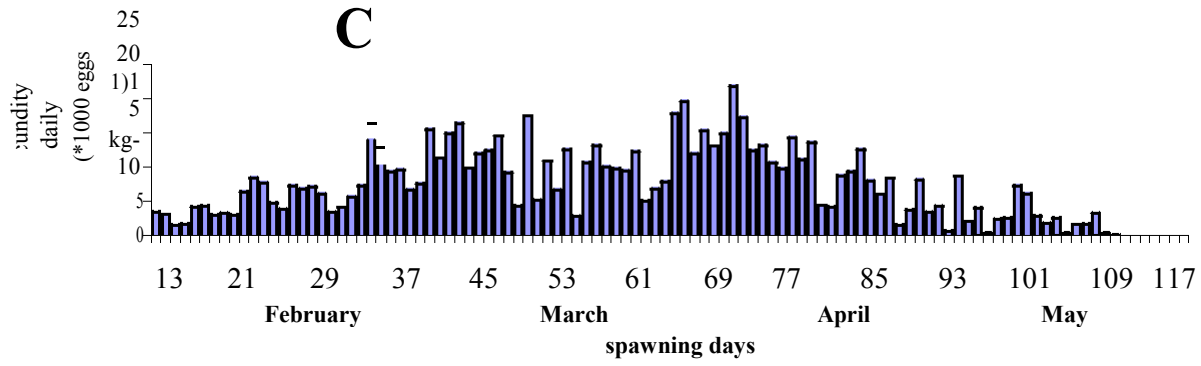
Spawning and eggs quality comparison results between the two breeding seasons were treated using the ANOVA test (analysis of variance) to a minimum of significance P <0.05. The existence of a correlation between eggs quality parameters was examined on the basis of simple regression. The results are expressed as means ± SEM. Statistical analysis was performed with the software Stat graphic 5, Plus

## RESULTS AND DISCUSSION

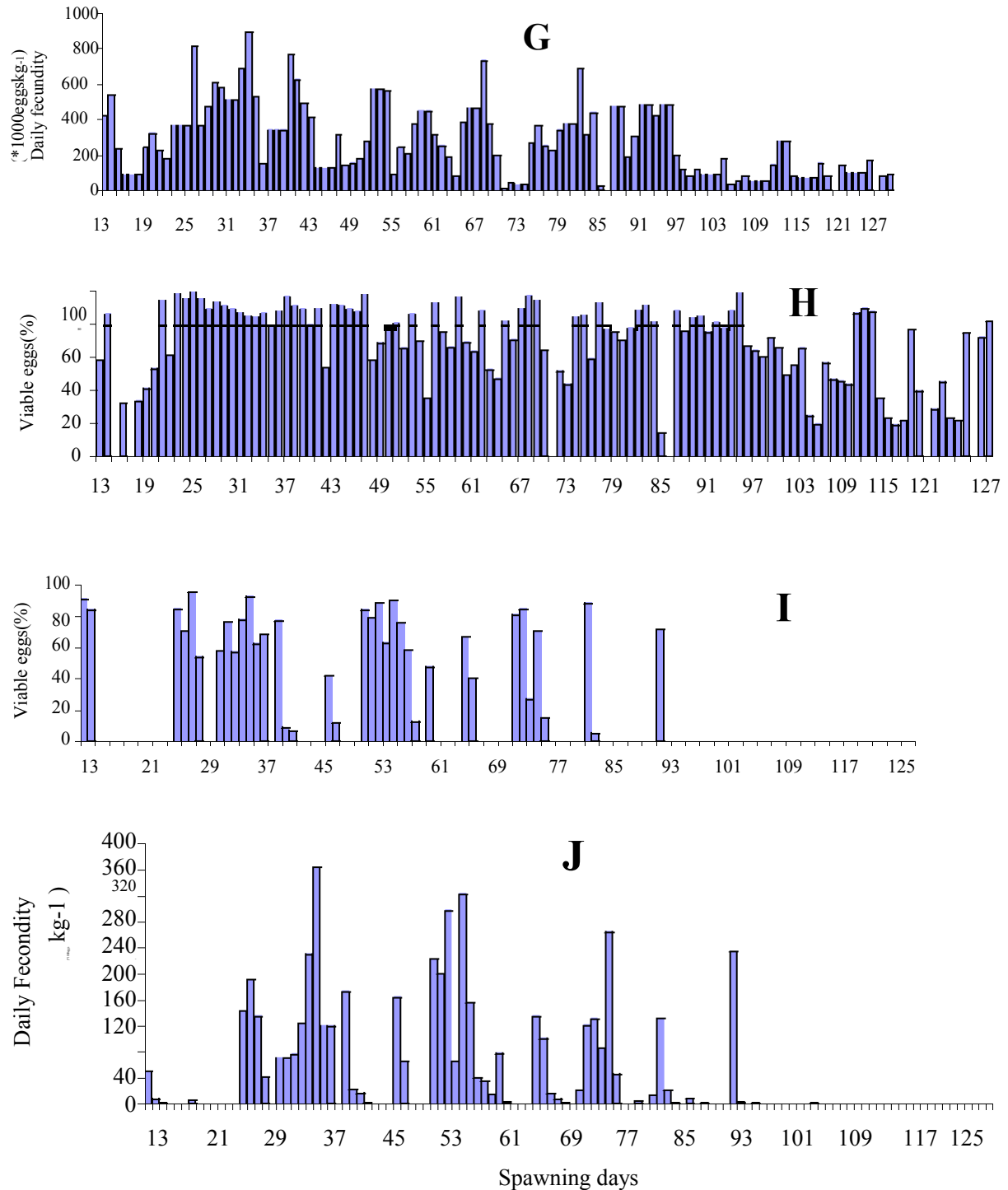
### Spawning and eggs quality

The spawning season of red porgy lasted from February 13 to May 25 during 2007 (Figure 1; C, D, E, F)) and 13 February to 06 June 2008 (Figure 2). Group A showed a significant decline in the middle of the spawning season in the second year (Figure 1).

Notable increases in the total number of daily collected eggs and fertility were observed in group B during the second year (2008) (G and H); the reproduction period for this broodstock group has been more prolonged up to 125 days (Figure 2). While spawning was daily, for group B, group A showed discontinuous spawning (I and J). The annual fecundity average has decreased significantly between the two years for group A (Figure 1), while it has



**Figure 1.** 2007 spawning; daily fecundity (group A: E; group B: C) and eggs viability rate (group A: D; group B: F).



**Figure 2.** 2008 Spawning. Daily fecundity (group A: J; group B: G) and eggs viability rate (group A: I; group B: H).

increased for group B in 2008 (Figure 2). Fertility showed a significantly difference ( $P < 0001$ ) between the two years. Statistically, There was a significant difference between the two years in the parameters examined (ANOVA,  $P < 0.05$ ).

Annual relative average fecundity varied from 474,000 to 192,000 eggs kg<sup>-1</sup> in group A and from 1,100,000

eggs to 769,000 kg<sup>-1</sup> in group B. The average weight of females of the two groups did not varied significantly between the two spawning seasons, because at the end of the first season, there was some mortality in both groups (Table 3).

There was a big difference of total eggs production between the two years within the same group and a huge

**Table 2.** Cumulative spawning of the two broodstock groups of common porgy during two consecutive spawning seasons (2007 - 2008).

Year	2007		2008		
	Group	A	B	A	B
Annual fecundity (1000eggs kg <sup>-1</sup> )		474.9 ± 8.5	769.2 ± 13.8	192.1 ± 3.8	1021.4 ± 20.4
Total eggs collected (*1000)		22,344.7	36,656.3	5,022.8	32,213.9
Viable eggs collected (*1000)		18,850.3	29,767.1	2,887.1	23,450.4
1-day embryo survival		53.82± 0.97	70.00 ± 1.26	75.43 ± 1.51	78.97 ± 1.58
Annual hatching rate		40.05± 0.72	49.65 ± 0.89	48.06 ± 0.96	67.58 ± 1.35
5-day larval survival		13.61±0.24	28.63 ± 0.52	37.71 ± 0.75	42.08 ± 0.84

**Table 3.** Females' biomass of the two broodstock groups during 2007 and 2008.

Year	2007		2008		
	Group	A	B	A	B
Number of females		14	13	10	11
Total females biomass (kg)		32.50 ± 0.65	35.58 ± 0.71	47.05 ± 0.75	47.66 ± 0.76
Average Weight (kg)		2.32 ± 0.04	2.74 ± 0.05	2.58 ± 0.05	2.8

difference between groups B and A. Such difference was shown through eggs quality parameters (C and D) and hatching larvae rate, 2<sup>nd</sup> and 5<sup>th</sup> d-larval survivals (Table 2). The regression analysis showed that there was a correlation between fertility and the rate of viable eggs for the two spawning seasons (C and D).

Similarly, there was a significant correlation between 2<sup>nd</sup> d-larval survival and viable eggs rate, and eggs hatching and 2<sup>nd</sup> d-larval survival during the two spawning seasons.

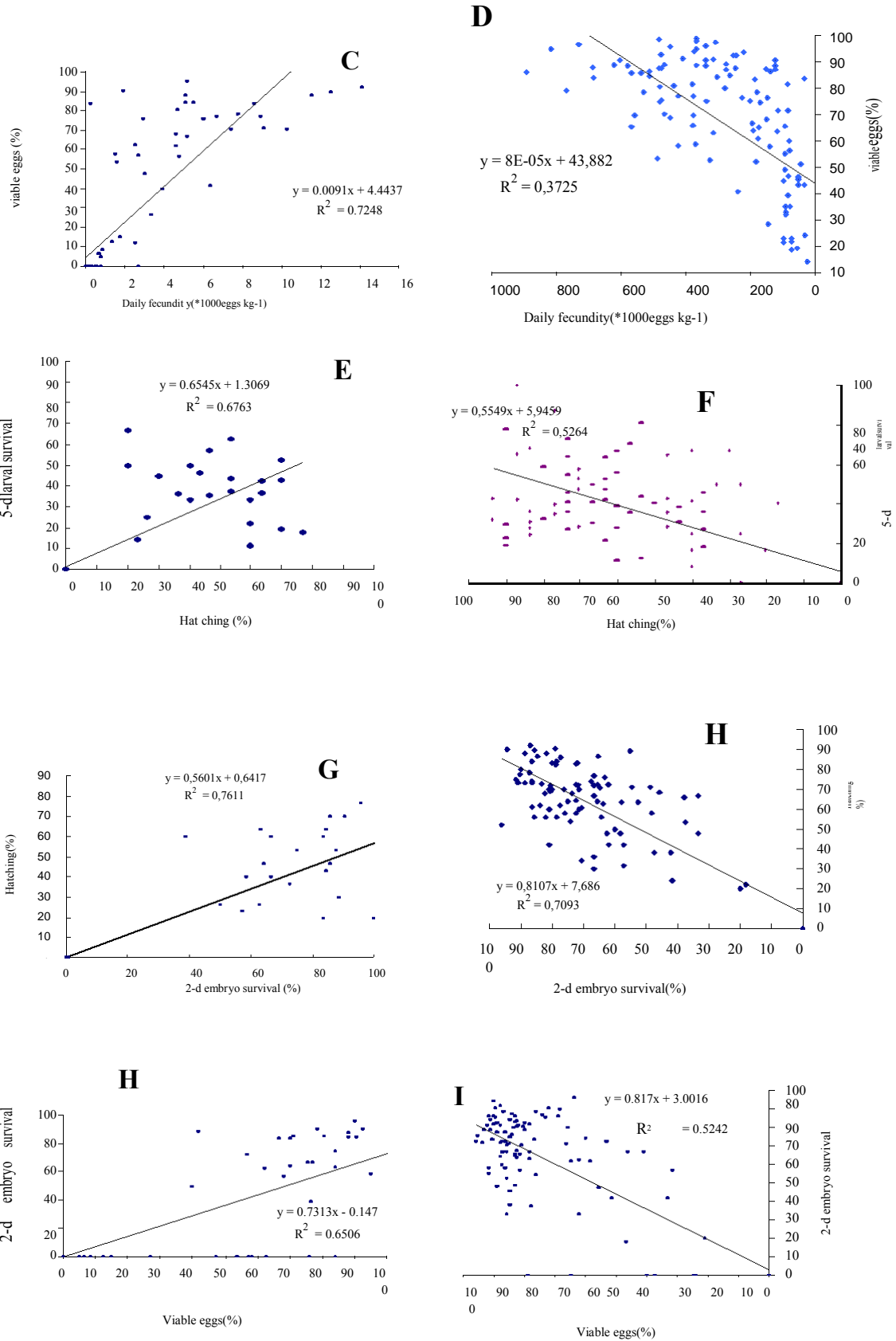
The relationship between hatching rate and 2<sup>nd</sup> larval survival still remained significant (G and H) with  $P < 0.03$  and  $P < 0.02$  during 2007 and 2008 seasons respectively. Finally, 5th d-larval survival was correlated significantly with the hatching rate for tow years (E and F) . However, eggs and oil globule diameters did not show significant changes during the two spawning periods. They were ranged respectively between 1.07 and 0.99 mm, and between 0.24 and 0.22 mm, over the two spawning seasons, the average diameter of eggs was  $1.02 \pm 0.02$  mm and the average of oil globule diameter was of  $0.228 \pm 0.004$  mm

## DISCUSSION

The captive reproduction period of red porgy was founded to be ranged in this study from mid-February to late-May in 2007 (Figure 1) and from mid-February to early June in 2008 (Figure 2). For group B parents, the breeding period was longer over two years than group A; the spawning season began at the same day for both two groups during the two years. In general, for wild populations, the reproduction season starts earlier, in spite of

the wide geographical distribution of this species. For example, in the Atlantic Ocean, the spawning season runs from February to May (Manoocher, 1976; Pajuelo and Lorenzo, 1996), while in the eastern Mediterranean, the spawning runs between February and late April (Vassilopoulos and Papaconstantinou, 1992). The spawning season of red Porgy in captivity is generally reported from mid-February to early June (Mihelakakis et al., 2001) and from mid-February to mid-May (Kolios et al., 1997).

Finally, using captive broodstock under the same conditions as the present work, Kokokiris et al. (2000) reported that the spawning takes place between March and May, while males are fluent from February (Kokokiris et al., 2001). In nature, reproduction of adult fish takes place in deep waters (50-150m) where temperature is lower and more stable during the year (14.5 - 16.5°C), (Machias et al., 1998). In the present work, the fish were reared at temperatures fluctuating between 15.5 and 17.9°C. It is possible that fish undergo rapid maturation and begin to spawn when water temperature begins to increase (Manoocher and Hassler, 1978), basically at the beginning of each winter. Similar spawning results of common porgy occurred in the Canary Islands (Mylonas et al., 2004) under water temperature ranged between 18 and 20°C (Pajuelo and Lorenzo, 1996). Daily fertility was significantly different between the two parents groups during the two consecutive years. The large number of spawning per year (Figure 1 and 2) produced by group B could not be attributed to the number of females constituting its broodstock. The two groups had indeed the same number of females, so results differences could be linked to parents' diet effect. Regarding the differences in spawning season duration between the two



**Figure 3.** Linear correlation between the different production parameters (Daily fecundity-viable eggs: C and D; hatching-5-d embryo survival: E and F; Hatching-2d embryo survival: G and H; viable eggs- 2d embryo survival: I and J). Correlations were very significant.

parents groups, the extended spawning period of group B in 2008 could be linked to food. The two parents groups have been kept in the same type of tanks, located side by side, provided by the same seawater source and exposed to same lighting conditions. The obtained values of the relative fertility were higher (especially for the group B, 1.1 million eggs kg<sup>-1</sup>) than reported values by Mihelakakis et al. (2001), 770,000 eggs kg<sup>-1</sup>. For group A, these values are almost identical to those reported in another study conducted by Kolios et al. (1997), 200,000 eggs kg<sup>-1</sup>. For similar cases, other fish species such as red sea bream, annual fecundity would vary between 260,000 eggs kg<sup>-1</sup> (Kafuku and Ikenoue, 1983) and 660,000 eggs kg<sup>-1</sup> (Watanabe and Kiron 1995). The annual fertility showed wide variations during the spawning season (Figure 1 and 2), particularly during the second year of monitoring. In 2008, group A fertility decreased to 59.54%, while in group B, it increased (32.78%) compared to 2007 spawning year. Generally, the average viable eggs rates were of 84.19 and 81.9% in 2007, and 57.3 and 72.5% in 2008 respectively for groups A and B.

These rates values corresponded to other studies of cultured red porgy. Kentouri et al. (1995) reported that spawning performances of two broodstocks over two consecutive years were ranged between 2 and 60% of viable floating eggs. In another study, a large broodstock of red porgy (of 100 kg female biomass) maintained at low densities in 42 m<sup>3</sup> outdoor tank produced eggs of mean fertilization success superior to 80% (Mihelakakis et al., 2001). In our study, an improvement in viable eggs rate was obtained towards the middle and declined towards the end of the spawning season. Moreover, it was observed an increase in the total number of viable egg production with an average of 400,000 in 2007 and 700,000 eggs kg<sup>-1</sup> in 2008 (Table 2). Group B showed improving results of eggs and larvae quality particularly. This improvement was mainly due to type and quality of food provided to group A similar effect was also observed in sea bream, common dentex (*Dentex dentex*) (Pavlidis, 2000). The observed varying egg and larval quality produced in fish farms of various species can be considered as an important limiting factor on aquaculture development (Kjørsvik et al., 1990; Bromage, 1995). The quality of the produced eggs can be influenced by various parameters which can change during the spawning season, such as feed ratio, feed quality, water temperature, water quality, handling, stress, etc. (Kjørsvik et al., 1990; Campbell et al., 1992; Bromage, 1995; Christiansen and Torrissen, 1997; Carrillo et al., 2000). It is useful, therefore, to be able to determine the quality of the produced eggs in a particular batch and predict the larvae survival, before investing facilities, manpower and time in incubating eggs and rearing larvae. Some of the egg quality markers employed in different fishes include fecundity, buoyancy of pelagic eggs, fertilization success, morphological characteristics (cleavage pattern or oil

globules distribution), eyeing percentage (salmonids) and hatching success (Kjørsvik et al., 1990; Bromage and Roberts, 1995; Brooks et al., 1997; Shields et al., 1997; Nocillado et al., 2000). In this study, correlations between different eggs productions and larval survival were examined. Based on obtained results, it was found that the rate of viable eggs had a statistically significant correlation with 2<sup>nd</sup> d of larval survival, which in turn was significantly correlated with hatching rate (Figure 3). Larval survival on 5<sup>th</sup> d was also correlated with hatching rate. These results suggested that larvae survival before exogenous feeding can be predicted, to some extent by viable eggs rate or by the 2<sup>nd</sup> d-larvae survival. Particularly with regard to the relationship between the hatching rate and 2<sup>nd</sup> d of larvae survival, it is important to note that batches of eggs with more than 60% in larval survival 2<sup>nd</sup> d had always more than 80% in hatching success. Various predictors of eggs quality have been identified in other species, for example, in the European sea bass (*Dicentrarchus labrax*) hatching success and 4<sup>th</sup> d larval survival exhibited a significant positive correlation with 1<sup>st</sup> d larval survival (Mylonas et al., 2003). The symmetry of cell division at the early blastula stage is considered as a strong predictor of hatching and normal larval development in cod, *Gadus morhua*, (Kjørsvik, 1994), halibut, *Hippoglossus hippoglossus* (Shields et al., 1997) and other marine fishes (Kjørsvik et al., 1990), including the red sea bream (Sakai et al., 1985). Finally, the easiest to apply and best studied egg quality determinant has been reported in salmonids, where fertilization success correlates very well with subsequent eyeing and hatching percentage, as well as larval survival (Bromage and Cumarantunga, 1988). Manooch (1976) first reported that red porgy eggs are transparent and spherical in shape; their diameters were of 0.8 - 0.9 mm and have a centrally located oil globule of 0.19 - 0.21 mm. Kolios et al. (1997) found mean egg diameter of 0.84 mm for fish kept in captivity at 13 - 25°C, whereas Mihelakakis et al. (2001) observed egg diameters from 0.99 to 1.09 mm during one spawning season. In our study, the eggs and oil globule diameters were ranged between 1.07 and 0.99 mm, and between 0.24 and 0.22 mm, respectively in the red sea bream. Normal eggs can range in diameter between 0.66 and 1.03 mm, without any reported effect on egg quality (Watanabe and Kiron, 1995). Unlike an earlier published report on red porgy (Mihelakakis et al., 2001), significant changes in oocyte diameters were not observed in the present study. A reduction in egg diameter, and therefore yolk content, may occur towards the end of spawning period in fishes with asynchronous ovarian development (Mihelakakis et al., 1995, 2001), and may result in a reduction in the embryo and larvae survival percentage (Blaxter, 1988). The above results show that we can expect a production of eggs for quality at any time during the spawning season of red porgy, if we introduce a healthy diet during this season.

## Conclusion

This work provides new information on spawning, egg production and quality characteristics of red porgy in captivity, and provides ample evidence on the effect of broodstock food on produced eggs quality and quantity during the spawning season of this species. This information is valuable for commercial hatcheries, particularly in regard to their requirements of broodstock biomass, spawning, egg production and predictors of eggs quality and larvae survival. Further research on the captive red porgy reproduction should focus on the effect of feeding broodstock, sex ratio and nutrition on egg production quality.

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