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The Effect of Temperature on Embryonic Development of the Red Porgy (*Pagrus pagrus*) Eggs

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Özet: ***Fangri (Pagrus pagrus) yumurtalarının embriyolojik gelişiminde sıcaklığın etkisi.*** Fangri (*Pagrus pagrus*) yumurtalarında sıcaklığın embriyolojik gelişim üzerindeki etkisinin saptanması için 10 (8, 10, 12, 14, 16, 18, 20, 22, 24 and 26°C) farklı sabit su sıcaklığı denenmiştir. Embriyonik gelişim 14-22°C'ler arasında tamamlanmıştır. Regresyon analizleri sonucunda, embriyonik gelişim ile inkübasyon sıcaklığı arasında negatif ilişki olduğu gözlenmiştir. 8 ve 26°C su sıcaklıklarında hücre bölünmesi meydana gelmemiştir. 10°C'de döllenmeden 5:45 dakika sonra 8 blastomer safhasında total ölüm gözlenmiştir. Bunun yanı sıra, 12 °C'de döllenmeden 42:45 saat sonra ½ gastrula safhasında ve 24°C'de döllenmeden 4 saat sonra erken blastula safhasında toplam ölümler tespit edilmiştir.

Anahtar Kelimeler: Fangri mercan (*P. pagrus*), yumurta, embriyolojik gelişim, sıcaklık.

Abstract: In this study, ten different constant water temperatures (8, 10, 12, 14, 16, 18, 20, 22, 24 and 26°C) were used in order to determine relationships on effects of water temperature on embryonic development of the red porgy (*Pagrus pagrus*) egg. Constant water temperatures between 14 and 22°C were found to support successful embryonic development. According to regression analysis, a negative relationship between the rate of embryonic development and incubation temperature was observed. While embryonic development was completed within this range (14-22°C), there was no cell division at water temperatures of 8 and 26°C. Total mortalities were observed at 8 blastomer stage in 10°C at 5:45 h after fertilisation. Also, in middle gastrula stage at 12°C, it was determined 42:45 h after fertilisation and 4 h in early blastula stage at temperature of 24°C after fertilisation.

Key Words: The red porgy (*P. pagrus*), egg, embryonic development, temperature.

Introduction

The red porgy *(Pagrus pagrus)* is one of the most highly estimated fish species in Mediterranean countries. It is a species intensively farmed in the Mediterranean (Basurco and Abellan, 1999) and has been targeted because of its fast growth under intensive farming conditions (Divanach *et al*., 1993; Stephanou *et al*., 1995; Kentouri *et al*., 1995).

Water temperatures during spawning and the incubation of the eggs are particularly important in affecting egg quality. Temperature may affect metabolism, activity and structure of the developing embryo (Kinne and Kinne, 1962). Although more studies have done on relationship between water temperature and salinity on embryonic development of red sea bream egg, there is lack of information in the red porgy (Machinandiarena *et al*. 2003). However, the relationship between incubation temperature and embryonic development stages of this species has yet to be described. The current study was undertaken to provide information about the effects of temperatures on duration of incubation period and morphogenesis of the red porgy eggs.

Materials and Methods

Broodstock were caught and acclimated for 2 years in a commercial hatchery Akuvatur Marine Product Inc., Izmir/Turkey. The fish were kept indoor polycarbonate 10 m^3 tank of 3 m diameter; water depth 1.2 m under natural photoperiod. Sex ratio was 6 females (1.2±0.3 kg mean body weight) and 12 males (0.7±0.2 kg mean weight). Sea water without treatment and thermal control was supplied continuously to the tank at the rate of some 2.5 m^3/h. Frozen cuttlefish (*Sepia officialis*) and shrimp (*Palaemon elegans*) were the primary food source and were provided daily. Manipulation and stripping of fish before and during the spawning season were avoided to reduce stress and injuries. After the first spawning took place, egg collectors of the broodstock tank was monitored and emptied every 15 minutes until the batch of eggs used in the study was obtained at 15:30 am on 27nd April 2004. The water temperature at spawning was 17.2°C. The eggs were washed carefully with fresh filtered seawater, and approximately 2000 fertilized eggs were placed in each incubator (glass beakers) of 1.5 l water volume. A total of 30 incubators were used in experiments. The diameter of 50 eggs was measured to the nearest 0.025 mm using an ocular micrometer

The experiment was carried out in triplicate at ten different constant temperatures (8, 10, 12, 14, 16, 18, 20, 22, 24 and 26°C). The eggs taken from egg collector of the broodstock tank were gradually adapted to each test temperature in 15 minutes. The adaptation duration was limited to 15 minutes to be able to observe egg development

from the stage of first cleavage at all test temperatures. As rapid adaptation may have caused were mortality, survival of the eggs was not a concern in this study. A minimum of ten eggs were removed for examination from one of the 3-glass beakers at each temperature at 15 min. intervals for the first 13 h, and thereafter then every hour (as in Jennings and Pawson, 1991). A description of egg development was recorded prior to fixation in 4% distilled water formaldehyde buffered to pH 7 with sodium acetate. In addition, eggs at 18°C were photographed at one-hour intervals throughout development. The temperatures of the glass beakers were maintained in a 75x40x25 cm aquarium filled with water heated by an electric heater or cooled by addition of cold water from a cooling system. The water in the aquaria was circulated by means of aeration. Temperatures in the glass beakers were recorded at hourly intervals with a calibrated thermometer, to a precision of ±0.05°C. Water flow rate was adjusted in a way to change 10% of the total volume of the beakers in an hour and dead eggs were removed immediately. Aeration rate was applied at a rate of 30 ml/min. Oxygen saturation was over 95%, salinity was 37‰ and pH was around 7.7. Ammonia and nitrite were always <0.010 mg/1.

The experiment was terminated when 50% of viable eggs at each temperature had hatched, or when all eggs at particular temperature were dead. Development was divided into a series of stages using descriptions from fresh eggs, examinations of preserved eggs and by reference to the photographic record. Stages were defined on the bases of 4 criteria by Jennings and Pawson (1991): 1. Stage description was based on unambiguously defined and clearly identifiable embryonic events. 2. Stages must appear to begin and end concurrently in fresh and preserved eggs of the same age. 3. Stages must change sufficiently frequently to provide the required precision of ageing. 4. Transition between stages must be short relative to stage durations, thus ensuring that most eggs examined may be assigned to specific stages. At the end of experiment, regression analyses of development time/mean incubation temperature were carried out for each group.

Results

Diameters of egg and oil globule were determined as 0.952±0.042 mm and 0.22±0.011.mm (mean±standard deviation) respectively. The temperature controlled aquaria maintained all temperatures within ±0.5°C of the mean of each row of glass beakers (Table 1). No unexpected data on water temperatures were recorded in beakers during this study.

Seven stages were selected to describe the red porgy egg development from fertilization to hatching, and the first of these stages was subdivided into six further stages based on cell numbers, as suggested by Simpson (1971), Nichols (1989) for plaice (*Pleuronectes platessa*) eggs, Jennings and Pawson (1991) for sea bass (*Dicentrarchus labrax*) eggs, to increase the precision of ageing. Stages are described in Tables 2 and Table 3. In addition, stages in Table 3 are shown with detailed photographs according to Jennings and Pawson (1991) due to more complex embryonic development than Table 2 (Figure 1):

Table 1. Incubation temperatures maintained in temperature controlled aquaria during the experimental incubation of the red porgy eggs (* 100% dead).

			Temperature (°C)			
Row		No. of hours running	Min.	Max.	Mean	SD
	1		8.1	8.4	8.21	0.031
I	2	-	8.0	8.3	8.19	0.042
	3		8.1	8.4	8.37	0.038
	1		9.7	10.1	9.91	0.056
II	2	5:45*	9.9	10.2	10.13	0.041
	3		9.6	10.1	9.87	0.076
	1		11.7	12.2	12.14	0.201
III	2	42:45	11.8	12.2	12.11	0.136
	3		11.9	12.4	12.04	0.111
	1		13.7	14.2	13.93	0.154
IV	2	82:30	13.7	14.1	14.01	0.123
	3		13.9	14.4	14.15	0.154
	1		15.7	16.4	16.18	0.324
V	2	66:15	15.8	16.5	16.07	0.189
	3		15.9	16.3	16.03	0.108
	1		17.6	18.3	17.94	0.277
VI	2	52:00	17.8	18.2	17.93	0.298
	3		17.6	18.4	18.12	0.237
	1		19.7	20.2	19.50	0.123
VII	2	42:30	19.8	20.3	20.13	0.109
	3		19.6	20.1	19.48	0.023
	1		21.8	22.1	22.02	0.167
VIII	2	33:30	21.8	22.3	22.01	0.167
	3		21.5	22.4	21.98	0.226
	1		23.6	24.2	23.72	0.176
IX	2	04:00*	23.5	24.1	23.65	0.138
	3		23.8	24.3	23.71	0.096
	1		25.82	26.3	26.1	0.243
X	2	-	25.91	26.4	26.2	0.191
	3		25.72	26.1	25.8	0.253

Eggs from all samples were assigned to these stages or, when in transition between stages (i.e. a/b, b/c, in Fig. 1), described as transitional. Times taken to reach stage midpoints following fertilization were calculated at each temperature, from the mean of egg ages during the transitional periods before and after age stage. When consecutive samples contained eggs from two different stages, but a transitional condition was not observed, the time of transition was estimated as a mean the two sampling times. While embryonic development was completed at 14, 16, 18, 20 and 22°C (Fig 3), there was no cell division occurred at water temperatures of 8 and 26°C. Total mortalities were observed at 8-clevage stage in 10°C at 5:45 h after fertilisation. Also, in middle gastrula stage at 12°C, it was determined 42:45 h after fertilisation and 4 h in early blastula stage at temperature of 24°C after fertilisation (Fig 2).

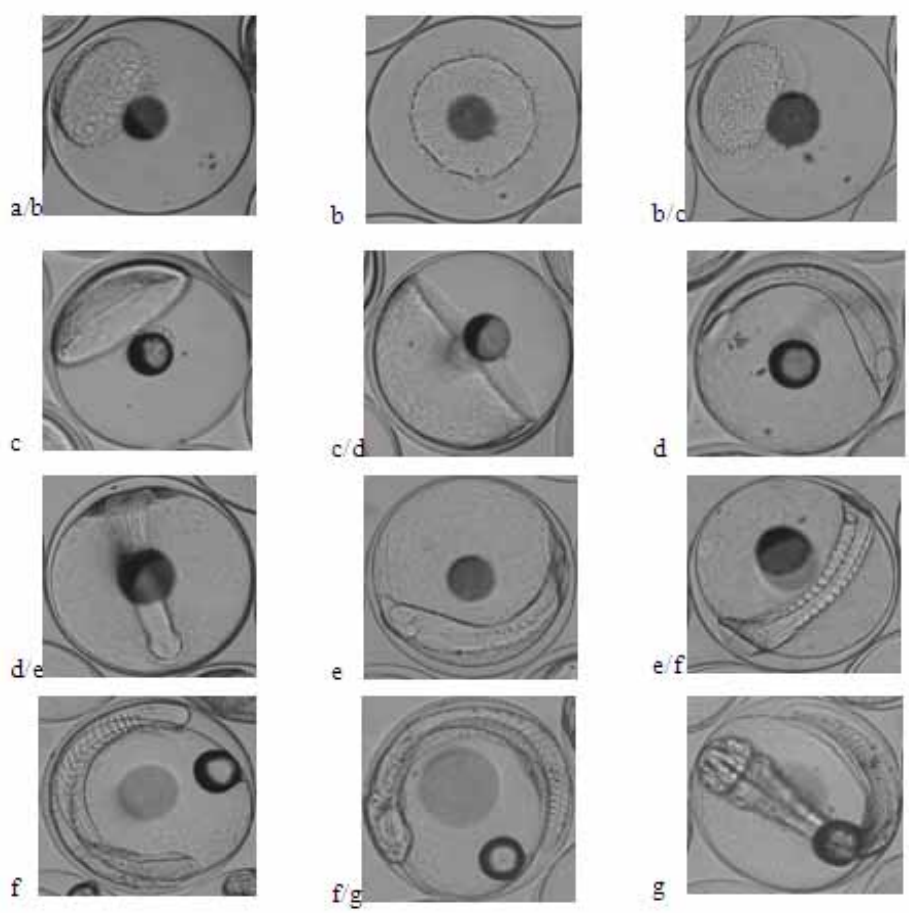

Figure 1. The embryonic developmental stages of the red porgy eggs at 18°C for Table 3.

Table 2. The identification keys of early developmental stages for the red porgy egg. In all cases stages start and end when complete membranes have formed through dividing cytoplasm.

Stage		Description
1	a	From the undivided egg cytoplasm until a complete membrane appears between 2 cells.
	b	From appearance of complete membrane between 2 cells until a complete membrane appears between 4 cells.
	c	From appearance of complete membrane between 4 cells until a complete membrane appears between 8 cells.
	d	From appearance of complete membrane between 8 cells until a complete membrane appears between 16 cells.
	e	From appearance of complete membrane between 16 cells until a complete membrane appears between 32 cells.
	f	From appearance of complete membrane between two 32 until a complete membrane appears between 64 cells.

Table 3. The identification keys of later developmental stages for the red porgy egg. In all cases stages start and end when complete membranes have formed through dividing cytoplasm (e.g. stage 2).

Stage		Description
2	a	From fertilization until the seventh division of cytoplasm begins to form approximately 128 cells (fresh material) or until individual cells can no longer be distinguished within the blastodisc (preserved material).
	b	Begins with completion of the seventh division of cytoplasm 128 cells (fresh material) or when individual cells can no longer be distinguished within the blastodisc (preserved material), Ends when the blastodisc margin begins to thicken and the germ ('signet') ring first appears.
	c	From the first appearance of the germ ring until has enveloped half the yolk mass.
	d	Begins with the germ ring enveloping half yolk mass, and ends with blastopore closure.
	e	From blastopore closure until the embryo extends 2/3 around the internal circumference of the egg.
	f	Begins with the embryo extending 2/3 around the internal circumference of the egg, and ends when it extends 3/4 around.
	g	From the embryo extending 3/4 around the internal circumference of the egg, until hatching occurs.

The relationships between temperature and egg ages (in hours) at stage midpoint were expressed by the relationship *ln age= a+b* temperature. Values of a and b were calculated by linear regression analyses to provide predictive equations that allowed ageing of eggs from observed stages and mean environmental temperatures (Table 4). Coefficients of determination, *r*, (Table 4), indicate that the regression analysis carried out provides reliable relationships between temperature and age for the seven main developmental stages and for the six subdivisions of stage I.

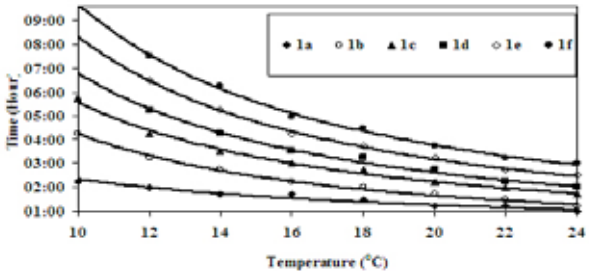

Figure 2. Developmental rate of dentex eggs in relation to temperature (Stage 1).

Table 4. Regression coefficients for the relationship between the natural logarithm of the red porgy egg ages (hours) and temperature (°C) at developmental stage midpoints (*ln age= a+b* temperature).

Stage		a	b	*r*
1	a	0.7284	-0.8685	0.97
	b	3.8566	-1.3372	0.99
	c	4.7037	-13057	0.98
	d	6.5953	-1.3634	0.99
	e	8.3521	-1.3814	0.96
	f	9.1248	-1.3547	0.99
2	a	255.92	-1.3795	0.99
	b	1764.5	-1.748	0.96
	c	3960.4	-1.9792	0.99
	d	5802.1	-2.0102	0.97
	e	7157.6	-1.8593	0.99
	f	10526	-1.9193	0.99
	g	15906	-1.9844	0.98

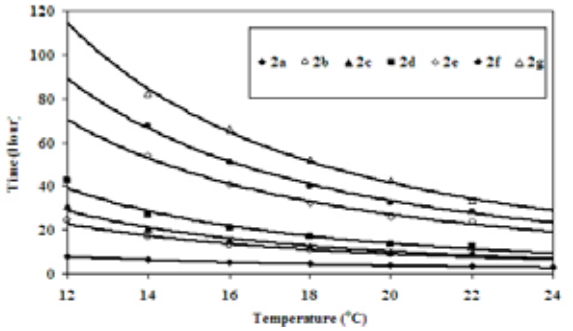

Figure 3. Development of the red porgy eggs in relation to temperature (Stage 2).

Discussion

Spawning of broodstock, embryo development, survival, and growth of fish larvae occur within a narrow range of water temperatures. Incubation temperature has a direct effect on the timing of embryonic development and thus determines hatch rate (Claireaux and Lagardere, 1999; Conides and Glamuzina, 2001). Fish development and hatching is delayed at low temperatures, and accelerated at high temperatures. However, cell division in egg is not completed except limited water temperature ranges. Incubating temperatures are also known to modify the behavior of larvae and determine certain morphological characteristics. As is usual for other species, the embryonic development of the red porgy investigated in this study is influenced by water temperature.

There is an optimum temperature required for each developmental life stage, and these vary among species. Optimal incubation temperature required for embryonic development of the eggs varies between the species. Gilthead sea bream (*Sparus aurata*) had optimal water temperature of 19°C with a range of ±3°C (Polo *et al*., 1991), which is a fairly wide one. For European sea bass, the optimal range is between 15 and 17°C (Conides and Glamuzina, 2001). For red sea bream (*Pagrus major*), successful hatching is observed in water temperatures between 14.5°C and 25.6°C (Mihelakakis and Yoshimatsu, 1998). The most successful hatching for sole (*Solea solea*) was observed between at water temperatures of 8°C and 12°C (Baynes *et al*., 1993). Machinandiarena *et al*. (2003), recorded that the emryonic development of red porgy egg was completed in 50 h at 18°C. In the present experiment, optimum temperatures for the development of the red porgy eggs have ranged from 16 to 18°C, although the lower and upper limits of hatching are 14°C and 22°C.

It is frequently difficult to maintain a suitable laboratory broodstock and obtain naturally fertilized eggs within minutes of release, and stripping wild fish is often the only option (Jennings and Powson, 1991). Therefore, many authors have relied on artificial fertilization to obtain eggs for developmental studies (Riley, 1974; Thomson and Riley, 1981). Eggs used in this study were obtained and transferred to the experimental beakers immediately after fertilisation. Embryonic development was monitored every fifteen minutes. In this way, development of the eggs in broodstock tank was prevented to avoid the development in the experimental temperature of 16.5°C. However, in order to clearly determine early stages at different temperatures, eggs were quickly adapted to experimental conditions in 15 min. Therefore, hatching and deformity rates are not presented in this paper due to the possible effects of direct thermal shock.

At temperatures below 8 °C, cleavage stage did not occur, and above 24°C cell division was either absent, highly asynchronous, or produced irregularly sized cells. This indicates that more than approximately 4°C changes from the water temperature of 16.5°C immediately after spawning may

drastically reduce the survival of the eggs. In this study at the water temperatures of 10, 12 and 24°C 100% mortality was observed before egg development was completed.

According to this study, there is a strong relationship between temperature and embryonic development of the red porgy eggs. However, a survival and deformation rate of the eggs for this species at various temperatures remains to be investigated. There are still some problems on obtaining of high amount of egg during the day time in partially spawner species. As a result of this, generally sufficient quantity of larva could not obtained for start of larval rearing in tanks. Finally, according to results of this study, it was supplied that, duration of embryonic development is manipulated on earlier and/or later by water temperature variations for hatching of egg on same time. Further investigations to improve the understanding of effects of other biotical and abiotical factors on embryology of this species may encourage its aquaculture as a highly commercial species.

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