Asian Journal of Chemistry Vol. 20, No. 4 (2008), 2603-2609

Effects of Some Heavy Metals on Hatching of Fertilized Eggs in Four Marine Fish Species in Aliaga Bay (Eastern Aegean Sea), Turkey

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The present study was carried out to determine the effects of some heavy metals such as Cd, Mn, Zn on hatching success of fertilized eggs of four marine fish species, *Dicentrarchus labrax* (Linnaeus, 1758), *Sparus aurata* (Linnaeus, 1758), *Dentex dentex* (Linnaeus, 1758), *Lithognathus mormyrus* (Linnaeus, 1758) in Aliaga Bay (Eastern Aegean Sea). The relationships between heavy metal concentrations in eggs and their hatching success were determined by multiple linear regression analysis. Results showed that magnitude and type of the effects of the three heavy metals studied in this research on egg hatching ratios differed based on fish species. In terms of the magnitude of the effect, Zn has a greater effect than Cd and Mn on the hatching in *D. dentex*, while it is smaller in *D. labrax*. The effects of Zn on egg hatching are in the same direction and about the same level for *S. aurata* and *L. mormyrus*.

Key Words: Heavy metal, *Dicentrarchus labrax*, *Sparus aurata*, *Dentex dentex*, *Lithognathus mormyrus*, Egg, Hatching success, Multiple regression, Aliaga Bay, Eastern Aegean Sea, Turkey.

INTRODUCTION

Fish eggs are crucial for continuity of population and vulnerable to heavy metal pollution. Heavy metal concentrations in seawater is effective on hatching success of fish eggs. Most of marine fish emit their eggs and spermatophores to marine water freely and fertilization occurs in seawater. In many countries, the substantial development of urban and industrial activities result in increasing inputs of chemical contaminants that leads to the loss or alteration of marine habitats[1-3]. When heavy metals in seawater exceed defined limits, they can affect the physiology of eggs and larva of

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marine fish[4,5]. This case is very important for continuity of natural populations and for hatcheries using seawater[6,7]. Because of their toxic affect to aquatic organisms, heavy metals such as cadmium, lead, chromium, nickel, copper, zinc and manganese[8,9] are in interest of researchers. Previous studies were focused generally on accumulation of trace metals in different tissues of sea fish such as liver, gills and muscle[10,11]. Karadede and Ünlü[12], studied the concentrations of heavy metals in the water, sediment and fish species (*e.g.*, *Acanthobrama marmid* Heckel, 1843; *Chalcalburnus mossulensis* Heckel, 1843; *Chondrostoma regium* Heckel, 1843; *Carasobarbus luteus* Heckel, 1843; *Capoetta trutta* Heckel, 1843 and *Cyprinus carpio* (Linnaeus, 1758)) from the Atatürk Reservoir in Turkey. Devauchelle and Coves[13] and Lahnsteiner *et al.*[14], studied the influence of physiological and biochemical parameters on egg composition of some commercial fish. The effects of chromium, lead and cadmium on the embryogenesis, hatching success and viability of the mallard were studied by Kertesz and Fanesi[15]. Metal concentrations in water and sediment with those in the muscle and livers of three species of fish caught in four seawater reservoirs (two in the Odiel estuary and two in the Bay of Cadiz) was comparatively studied by Usero *et al.*[6].

However, there is no detailed published report regarding the effects of heavy metals such as Cd, Mn and Zn in eggs on incubation success of eggs of commercial marine fish. Industrial wastes from Aliaga Oil Refinery have created a potential source of heavy metal in Aliaga Bay that is placed in the Eastern Aegean sea. Up to date, the relationships between heavy metal concentrations in eggs and hatching success have not been studied in Turkey.

This study aims to determine the influences of heavy metals as Cd, Mn and Zn on hatching success of fertilized eggs are belonging to four different marine fish *D. labrax, S. aurata, D. dentex* and *L. mormyrus* captured in Aliaga Bay which is located at coasts of the eastern Aegean sea.

EXPERIMENTAL

The brood stock samples of Sea bass, *D. labrax*; sea bream, *S. aurata*; dentex, *D. dentex* and striped bream, *L. mormyrus* used were collected by local fishermen in Aliaga Bay, Izmir province located in the Eastern Aegean sea. The brood stocks were purchased in the same day of capture and brought to the hatchery in Aliaga town. They are stocked in four circular polyester tanks of 12 m^3 as 2/1 (male/female) in stock density of 3 kg/m^3.

The eggs belonging to specimens of four different marine fish species were obtained from brood stocks during their natural spawning periods. The eggs of *D. labrax*, *S. aurata*, *D. dentex* and *L. mormyrus* were taken for analyses as soon as fertilization occurred. The sinking unfertilized eggs were removed and discarded. A total of nine aquariums for each species were used to determine the hatching success of the eggs.

Heavy metal analysis: The fertilized eggs of each fish species were pooled in nine Petri dishes as replicates. To dry the eggs, they were put into an oven set to 110 ºC. After the eggs reached constant weights in the oven they were transferred into digestion flasks. Perchloric acid (4 mL) and nitric acid (8 mL) were added and the digestion flasks were put on a hot plate that was gradually increased to 120 ºC. The digestion flasks were kept on the hotplate until all the eggs were totally dissolved. The digests were diluted with distilled water appropriately in the range of standards that were prepared from stock standard solution of the metals. Metal concentrations were measured using an Ati Unicam 929 AA spectrophotometer and metal concentration in eggs were presented as mg × metal/g dry weight.

Statistical analysis: Relationships between hatching success and heavy metal *e.g.*, Zn, Mn and Cd were investigated *via* multiple regression analysis[16]. To obtain the identical unit, the variables were standardized and the relations among the variables were investigated by using of the model given in eqn. 1.

$$Yi = b_1X_{i1} + b_2X_{i2} + \ldots\ldots\ldots\ldots + b_pX_{ip} \quad (1)$$

where, Y = hatching success of eggs or dependent variable, X_i are the independent or predictor variables (i = 1, 2, 3), bi's are the standardized partial-regression coefficients. Each partial regression coefficient represents the direct effect the ith variable has on the dependent variable, holding the remaining X's in the equation constant[16]. It is possible to get information about the effect of each heavy metal on egg hatching by using standardized partial regression coefficients[17]. Statistical analysis of the data was carried out using the statistical package program SAS (Ver 8.0)[18]. Normality of distribution was tested for the variables with Shaprio-Wilks test before regression analysis, which showed that all variables were normally distributed except Zn in dentex and Zn in seabass. These two varibles were analyzed after logaritmic transformation.

RESULTS AND DISCUSSION

In sea bass in estimates that are to be maked regarding egg hatching rates by a regression equation stated as DIC = -0.983 Zn +0.312 Mn -2.99 Cd (R^2 = 98.2 %, P = 0.068). Based on the regression equation for each one standard deviation increment in Zn, 0.983 standard deviation decrease is expected for egg hatching ratio; while one standard deviation increment in Cd would result in 2.99 standard deviation decrease. Meanwhile, for each one standard deviation increment in Mn, 0.312 standard deviation increase is expected for egg hatching ratio. As noticed, Zn and Cd had negative effects on egg hatching, while Mn had a positive effect. Cd (-2.99) seems to be the most effective heavy metal on the egg hatching ratio of seabass.

When the hatching rates in giltheads are estimated by using the equation SPA = 0.667 Zn -1.18 Mn +1.65 Cd, determination coefficient is 96.8 % (P = 0.071). Based on the regression equation: SPA = 0.667 Zn -1.18 Mn +1.65 Cd, Cd appears to be the most effective heavy metal on the egg hatching ratio in sea breams species. In this case, Zn and Cd have positive effects on egg hatching, while Mn had a negative one.

Consequently, for one standard deviation increment in Zn level, 0.667 standard deviation increase is expected in egg hatching ratio and one standard deviation increment in Cd would cause 1.65 standard deviation increase. For Mn, one standard deviation increment would cause 1.18 standard deviation decrease.

When the hatching rates in sea bream are estimated by using the equation DEN = -3.740 Zn +1.63 Mn -2.23 Cd, determination coefficient is 98.6 % (P = 0.047). In individuals of striped bream Zn (-3.74) is heavy metal most effected the hatching rates and follows by Cd (-2.23) and Mn (1.63). As the results it can be said that, while Zn and Cd decrease hatching rates, Mn increases hatching rates. Mn has the greatest effect on the hatching rates of stiped bream.

Using the regression equation LIT = 0.508 Zn -0.722 Mn +0.343 Cd, accuracy of the estimation for egg hatching ratio of L. mormyrus is R^2 = 99.1 % (P = 0.018). For this species, Zn (0.508) and Cd (0.343) have positive effects on hatching, while Mn (-0.722) has a negative effect.

Magnitude and type of the effects of the three heavy metals studied in this work on egg hatching ratios differed based on fish species. For instance, the effect of Zn is in negative direction on DIC and DEN, but in positive direction on SPA and LIT species. In terms of the magnitude of the effect, Zn has a greater effect than Cd and Mn on the hatching in DEN, while it is smaller in DIC. The effects of Zn on egg hatching are in the same direction and about the same level for both SPA and LIT species. Looking at the effect of Mn on hatching ratios, it has a positive effect on DIC and DEN species while a negative effect on SPA and LIT. However, Cd has a negative effect on DIC and positive effect on SPA. Also, it has a negative effect on DEN and a positive effect on LIT. Different directions and levels of the effects by heavy metals investigated here, may be attributed to the difference in families which these species belong to family, Sparidea and Seranidea, as well as the difference in their reproduction periods (spring and summer).

Although both essential and non-essential metals may retard fish development, in present study Zn and Cd seem to be more effective than Mn. This case is more obvious in *D. labrax, S. aurata* and *D. dentex*.

In this study, as the spawning periods of species are different, the values of physico-chemical parameters of seawater are also measured differently (Table-1). In this study, these values are found as 17 ºC for sea bass, 18 ºC gilthead, 18 ºC striped bream and 25 ºC dentex.

TABLE-1
PHYSICO-CHEMICAL PARAMETERS OF THE WATER IN THE COLLECTORS THAT HATCH EGGS OF THE SPECIES *Dicentrarchus labrax, Sparus aurata, Dentex dentex* AND *Lithognathus mormyrus*

Water parameters	*D. labrax*	*S. aurata*	*D. dentex*	*L. mormyrus*
Salinity (%)	36.6-37.2	36.7-37.4	36.9-37.2	37.2-37.4
Temperature (ºC)	17	18	18	25
Dissolved oxygen (mg L^{-1})	6.4-7.2	6.8-7.8	6.4-7.2	6.3-6.1

TABLE-2
MEAN CONCENTRATIONS (mg metal/g d.w.) OF METALS, HATCHING SUCCESS AND STANDARD ERROR OF MEAN IN THE EGGS OF THE SEA BASS, *D. labrax*, GILTHEAD, *S. aurata*, STRIPED BREAM, *D. dentex* AND DENTEX, *L. mormyrus* CAUGHT FROM THE EASTERN AEGEAN SEA (n = 12)

Variables	*D. labrax*	*S. aurata*	*D. dentex*	*L. mormyrus*
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Hatching success	83.65 ± 0.36	86.33 ± 0.58	83.83 ± 0.29	71.83 ± 0.65
Zn	104.90 ± 0.59	55.68 ± 1.26	52.45 ± 0.47	114.51 ± 2.00
Mn	3.46 ± 0.09	7.39 ± 0.09	7.79 ± 0.08	4.42 ± 0.18
Cd	2.71 ± 0.07	3.52 ± 0.07	1.93 ± 0.02	4.03 ± 0.09

As known, the relations between hatching success of eggs and heavy metals vary in respect of fish species (Table-3).

TABLE-3
STANDARDIZED PARTIAL REGRESSION EQUATIONS AND THEIR R^2 (%)

Species	Standardized partial regression equation	R^2 (%)
D. labrax	DIC = -0.983 Zn +0.312 Mn -2.99 Cd	98.2
S. aurata	SPA = 0.667 Zn -1.18 Mn +1.65 Cd	96.8
D. dentex	DEN = -3.740 Zn +1.63 Mn -2.23 Cd	98.6
L. mormyrus	LIT = 0.508 Zn -0.722 Mn +0.343 Cd	99.1

Heath[19] indicated that fish development can be affected by the presence of heavy metals in water and especially the early life stages such as hatching time, larval development and juvenile growth as they are more sensitive than the mature stages. Up to date, the relationships between larvae performance and egg diameter have not been found. But, this situation has been discussed for sea fish, tuna fish and freshwater fish[20,21]. For that, it can be accepted that the egg diameter is not a feature determining the larvae quality.

Rana[22] indicated that egg size has a less effective at the beginning of feeding. Devauchelle[23] reported that the hatching ratio in eggs of sea bass in 13-16 ºC was 72 %. The reason for this hatching ratio is lower than present levels (83 %) can be assumed that the incubation temperature in this study fluctuates highly (Table-2). The same present situation is valid for gilthead, dentex and striped bream and so living percentages in different temperatures can be also determined. Zn, Mn and Cd can change in terms of environmental conditions[13]. The values of temperature used in hatching periods of some sea fish are 19 ºC for 15-17 ºC sea bass[24], 14.5-55.6 ºC common sea bream[25] and 23-25 ºC dentex[26] in turns.

The diameters of eggs used in this work are 1.183 mm for sea bass, 1.14 mm gilthead, 1.05 mm striped bream and 0.80 mm dentex in turn (Table-4). The diameters of egg and lipid droplet resemblance to the other Sparids. Barnabe and Rene[27], measured as the diameters of egg 1.15-1.20 mm and lipid droplet 0.32-0.35 mm in sea bass, Katavic[28], as 1.07-1.24 mm; 0.35-0.40 mm finally, Rafaelle[29], 1.15-1.16 mm; 0.33-0.36 mm, respectively.

TABLE-4
DIAMETER OF EGG AND LIPID DROPLET

	Eggs and the diameters of lipid droplet			
	D.labrax	*S. aurata*	*D. dentex*	*L. mormyrus*
Egg diameter	1.183 ± 0.026	1.140 ± 0.064	1.054 ± 0.06	0.804 ± 0.0300
Diameter of lipid droplet	0.330 ± 0.022	0.224 ± 0.044	0.273 ± 0.03	0.186 ± 0.0072

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(*Received*: 22 February 2007; *Accepted*: 1 January 2008) AJC-6156