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Study of the Relationship Between Some Biological Characteristics of Ovum, Hydrated Eggs and the Age of "*Rutilus rutilus caspicus*" in Gorgan Gulf

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Abstract: During the years of 2002-2004, some biological characteristics of ovum and hydrated hydrated eggs were studied on 154 pieces of emigrant population of *Rutilus rutilus caspicus*, having 4 age classifications, in Gorgan gulf. The diameter of ovum and the ratio of its surface to its volume ranged from 0.94 mm to 1.27 mm and from 4.96mm^{-1} to 6.37mm^{-1} and in hydrated eggs ranged from 1.95 mm^{-1} to 2.54 mm^{-1} and 2.36 mm^{-1} to 3.16 mm^{-1}, respectively, which have a significant different between the diameter of ovum and hydrated eggs in different age classifications ($p<0.05$). The maximum and minimum of this difference were calculated in the age classes 4^{+}-5^{+} and 8^{+}-9^{+}. The ratio of the surface to the volume in hydrated eggs was significantly lower than the same ratio in ovum ($p<0.05$). Generally, this ratio decreased after increase in the diameter of the ovum, so that in the age class 8^{+}-9^{+}, comparing with other age classes, this ratio was quite significantly higher ($p<0.05$). The yolk sac space and the space around the yolk sac, respectively, occupy 49% to 60% and 40% to 51% of the hydrated eggs volume. The maximum of this figure was significantly calculated in the age class 4^{+}-5^{+}, which decreased after a decrease in the diameter of the hydrated eggs ($p<0.05$).

Key words:*Rutilus rutilus* · Biological features of ovum and hydrated eggs · Spawning fish · Fertilized ovums · Age classifications

INTRODUCTION

The "*Rutilus rutilus*" species is a member of the Cyprinus carpio family, its maximum length is 46 centimeter and its maximum weight is 1840 grams and the maximum reported age for it is 14 years. "*Rutilus rutilus caspicus*" is native to Caspian Sea [1]. It, during its life, breeds 5 to 6 times in temperatures 10 to 20 degree centigrade. The maximum of its emigration and spawning have been observed in temperature 14 to 18 degree centigrade and in the maximum depth of 50 m. however the reproduction emigration of this subspecies begins in 6 degree centigrade [2].

These fish have high transmittals in Caspian Sea and have important economical value in aquaculture [3]. Due to excessive fishing and also decay of many of natural spawning grounds, this species have included in endangered group's lists [4]. On the other hand the amount of annual fishing of this species has extremely decreased, So that sometimes, it has even affected on the amount of fishing of breeders with the purpose of artificial reproduction (excluding 2004). Therefore protecting the resources of this species is inevitable; accordingly this important problem has been solving through the artificial reproduction [5].

Because of the lack of information related to the characteristics of the female sex (size, age and condition), the relationship between maternal characteristics and the hydrated hydrated eggss has been less studied [6]. The reproduction potential in brood stocks is dependent on both biomasses (the index for the whole number of produced hydrated hydrated eggss) and the age combinations [7-10]. Different in dimensions of hydrated hydrated eggs of a species is related to spawning season [6, 11] the size of fishes [7-10], brood protection [6], absolute fecundity [12] and environmental factors [13]. The diameter of the hydrated eggs may affect on survival, larval size, nourishing activities, resistance to starvation and avoidance of hunters. However there is little information about the relationship between maternal characteristics such as age and size and other biological features have not been measured [6] so that in this

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situation we are not able to establish on authentic phylogenic relationship between fishes, because in most studies only the diameter of the hydrated eggs has been investigated and other biological features have not been measured [11].

The ratio of the surface to volume in hydrated eggss, affects directly on the metabolic rate of embryo and transferring different gases including oxygen. This ratio varies from fish to fish [11, 14]. The larval size increases by the increase in the diameter of the hydrated eggs. However sometimes it has been reported that those spawning fishes whose hydrated eggs have higher diameters, have smaller larvae, this could be due to small yolk sac and accordingly big space around the yolk sac. The spawn consist of the yolk sac and the space around the yolk sac. The larval size is directly related to the yolk sac size; by the increase in yolk sac size, larval size will increase. Therefore, in all studies we have measured and determined not only the diameter of the spawn but also the ratio of the spawn surface to its volume, the yolk sac size, the size of the space around the yolk sac and the ratio of the yolk sac size to the size of the space around it [11].

This study has been done on "*Rutilus rutilus*" for determination of the phylogenic of "*Rutilus rutilus*" among other species and using more suitable indexes for the diameter of the yolk sac, the ratio of the surface to the volume and the diameter of the hydrated eggs.

MATERIALS AND METHODS

From the middle of January in 2002 to the middle of April in 2004, 154 *Rutilus rutilus* female fishes were fished by the seine cooperative settled in the Strait of Gorganroud River with 20 to 30 mm mesh size. Firstly, ages of all samples were determined. They were classified into four classes including 4^{+}-6^{+}, 6^{+}-8^{+}, 8^{+}-10^{+} and 10^{+}-12^{+}. In order to determining age of each fish, 15 scales were scraped from the left body, between the dorsal fin and the lateral line. Then samples were transferred to the Central Laboratory of Agricultural Sciences and Natural Resources University of Gorgan for future analysis. After washing scales with water and soap, they were put between two lam, then ages were classified under oculometer loop [5]. From each age classes, 6 female fishes were chosen quite randomly and through the method introduced by [15], the brood stocks were intramuscularly injected with the hormone acquired from pituitary gland of carps (3 mg kg^{-1} body weight). Fishes were injected two times with 12 hours interval (10% and 90% of total dose, respectively) then reproduced. According to this method, at first for the purpose of determination of mature level of fishes, a few hydrated eggs, through biopsy and by using a catheter were expelled from 10 spawning fishes. These hydrated eggs, regarded their thick chorion and were kept for 10 minutes in a solution of 60% ethanol, 30% formalin and 10% glassial citric acid. Then under a loop equipped with optical micrometer, their nucleus positions were determined.

100 ovums, immediately after breeding and 100 hydrated eggs after fertilization and hydration (in petivitelline stage, [11] were sampled of each fish. After fixing these samples in glycerol solution (100 milliliters alcohol (60%), 88 milliliters water, 15 milliliters nitric acid (80%), 18 milliliters acetic acid and 20 milligrams mercury chloride) the diameter of these hydrated eggss were determined using a loop equipped with optical micrometer [6]. In order to calculate the ratio of the surface to the volume the following formulas can be used:

$$S= 4\pi r^2 \qquad V= 4.3\ \pi r^3$$

In above formulas S, V and r respectively refer to the surface, the volume and the radius of the hydrated eggss. Furthermore the diameter and the size of the yolk sac were calculated through the mentioned formulas. To calculate the ratio of the yolk sac to the space around yolk sac, at first the space around the yolk sac was calculated according to the following formulas:

$$Ps= V-Ys$$

In this formula Ps, V and Ys respectively refer to the space around the yolk sac, volume of the hydrated eggss and the yolk sac size divided by the space around it equals the mentioned ratio [11]. After calculating the biological features of the hydrated eggs of "*Rutilus rutilus*", in order to investigate the phylogenic relationship in this species, it was compared to other species, according to the extracted references [11].

In different age classes, in order to compare the diameter of the ovum to the diameter of hydrated eggs (millimeter) following factors were considered: the ratio of tow factors including the ratio of the surface to the volume of the hydrated eggs and the same ratio of the ovum, the ratio of the yolk sac size to the space around it, a quite random design in the form of Duncan test and one way analysis variance in spss 10.05 program.

The tables have been drawn using Excel 2000 program. The relationship between age and diameters of the spawn and the ovum (millimeter) has been offered using the regression equation.

RESULTS AND DISCUSSION

The quantities (mean, standard deviation) of some biological features of 2400 ovum and 2400 hydrated eggs of "*Rutilus rutilus*" species have been briefly presented in tables 1 and 2. In all cases quantities for hydrated eggs are more than those for the ovum and there is a significant difference ($P<0.05$) between them. This fact corresponds to results other researchers obtained [9, 10].

Because of water absorption by hydrated eggs and also because of the formation of the space around the yolk sac, the diameter of the hydrated eggs increases [10]. According to the study by [5] on turkman "*Rutilus rutilus*" in Gomishan lagoon, the mean of the diameter of the ovum in this species, after the increase in the sexual maturation, will gradually increase; this quantity will be maximum in the end of April. They found a significant correlation between total length, body weight, scale radius and age ($p<0.001$). The maximum growth rates were found in ages 1^+ and 2^+. The hydrated eggs diameter was measured with a range from 0.9 mm to 1.45 mm and the absolute fecundity was related to age. In the current study, the diameter of the ovum of "*Rutilus rutilus*" is sometimes between 0.96 to 1.48 millimeter; which corresponds to other studies [2, 5] and slight difference in our work with other researchers is probably because of different fish sizes in various studies [5].

In the table 2 the regression equations and diameters of ovum and hydrated eggs (millimeter) have been presented briefly. According to the high correlation we can say that there is a meaningful relationship between mentioned parameters. Furthermore, the quadratic equation shows that there is an alometric relationship between the age and the diameters of ovum and hydrated eggs (millimeter); which correspond to other research's studies [7-10]. The regression relationship varies according to species, sexes, different seasons, resources of the same species and different places. Therefore, a slight variance in regression equation is normal [5].

Table 1: The quantities (mean, standard deviation) of some biological features of ovum and hydrated eggs of "*Rutilus rutilus*" species in Gorgan gulf

	Diameter (millimeter)		Surface (square millimeter)		Volume (cubic millimeter)		hydrated eggs surface to its volume	hydrated eggs yolk sac to space around yolk sac
Factor	total	Yolk sac	total	Yolk sac	total	Yolk sac		
ovum	1.29±0.05[b]	-	4.4±0.30[b]	-	0.8±0.08[b]	-	5.52±0.23[b]	-
hydrated eggs	2.28±0.10[a]	1.17±0.05[a]	16.44±1.42[a]	4.33±0.33[a]	6.34±0.79[a]	3.63±0.51 [a]	2.65±0.12[a]	1.33±0.12[a]

Table 2: Regression equations and correlation between diameter of ovums and hydrated eggs (millimeter) with age of "*Rutilus rutilus*" in occasion study

Numbers	Relationship	regression equations	correlation
2400	Diameter of ovum-age	$Y=-297.71\ X^2+630.32X+327.17$	0.75
2400	Diameter of hydrated eggs-age	$Y=-71.566\ X^2+315.84X+341.98$	0.66

Table 3: Variance analysis of diameters of hydrated eggs and ovum (millimeter), the ratio of ovum and hydrated eggs surface to their volume and the ratio of hydrated eggs yolk sac to space around yolk sac in the age classes of "*Rutilus rutilus*" in occasion study

Factor		df	Sum of Squares	Mean Square	F	Sig.
Diameter of ovum	Between Groups	3	0.041	0.013	44.836	0.0005
	Within Groups	20	0.006	0.0003	-	-
	Total	23	0.047	-	-	-
Diameter of hydrated eggs	Between Groups	3	0.204	0.068	53.069	0.0005
	Within Groups	20	0.026	0.001	-	-
	Total	23	0.229	-	-	-
The ratio of ovumsurface to its volume	Between Groups	3	1.043	0.348	45.16	0.0005
	Within Groups	20	0.154	0.008	-	-
	Total	23	1.197	-	-	-
The ratio of hydrated eggs surface to its volume	Between Groups	3	0.301	0.100	51.054	0.0005
	Within Groups	20	0.039	0.002	-	-
	Total	23	0.341	-	-	-
The ratio of hydrated eggs yolk sac to space around yolk sac	Between Groups	3	0.103	0.034	3.221	0.045
	Within Groups	20	0.212	0.011	-	-
	Total	23	0.315	-	-	-

Table 4: Comparing means of the diameter of hydrated eggs and ovum (millimeter), the ratio of ovum and hydrated eggs surface to their volume and the ratio of hydrated eggs yolk sac to space around yolk sac in the age classes of "*Rutilus rutilus*" in occasion study

Factors	4^+ - 6^+	6^+ - 8^+	8^+ - 10^+	10^+ - 12^+
The diameter of ovum	1.34 [a]	1.32 [b]	1.23 [b]	1.29 [c]
The diameter of hydrated eggs	2.37 [a]	2.33 [a]	2.13 [c]	2.29 [b]
The ratio of ovum surface to its volume	5.30 [d]	5.41 [c]	5.85 [a]	5.54 [b]
The ratio of hydrated eggs surface to its volume	2.55 [c]	2.59 [bc]	2.84 [a]	2.64 [b]
The ratio of hydrated eggs yolk sac to space around yolk sac	1.44 [a]	1.33 [ab]	1.28 [b]	1.28 [b]

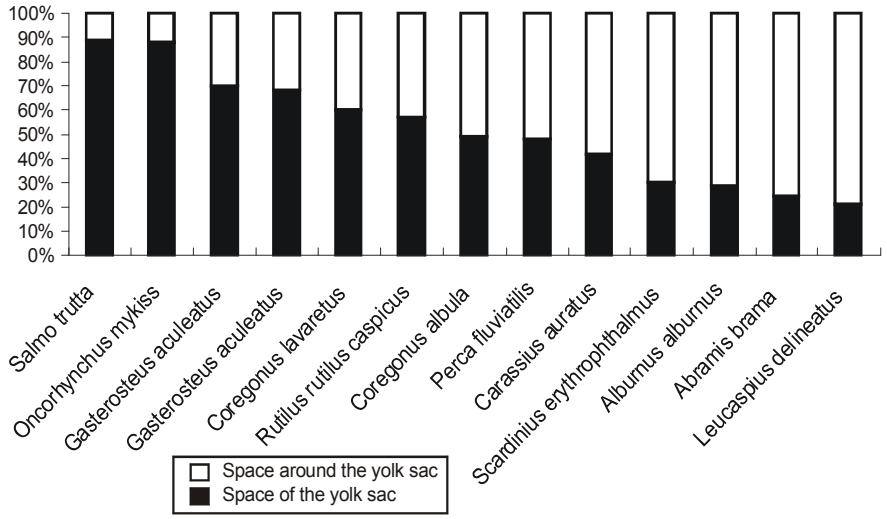

Fig. 1: The ratio of yolk sac size to the space around it in some fish species and emigrant population of "*Rutilus rutilus*" to Gorgan gulf in occasion study

In this study the maximum age, the total height and the total weight of "*Rutilus rutilus*" have been respectively calculated as 11^+, 403 millimeters and 437.62 grams. The biggest "*Rutilus rutilus*" which was formerly fishes in Gomishan lagon, was a female eight-year old "*Rutilus rutilus*" with 323 millimeters height and 477.2 grams weight [5]. According to variance analysis (Table 3) and after comparing means (Table 4) of diameters of hydrated eggs and ovum (millimeter), the ratio of surface to volume in ovum and hydrated eggss and the ratio of yolk sac to the space around it in different age classes, there is a significant difference ($P<0.05$) between mentioned parameters in different ages of "*Rutilus rutilus*"; so that the maximum ratio of yolk sac size to the space around the yolk sac have been reported in age class 4^+-6^+ and the maximum ratio of surface to volume in both ovum and hydrated eggs have been reported in age class 8^+-10^+. The minimum diameter of ovum and hydrated eggs (millimeter) and the minimum ratio of yolk sac size to the space around it have been reported in age class 8^+-10^+. The minimum ratio of surface to volume in both ovum and hydrated eggs has been calculated in age class 4^+-6^+. According to table 4, in "*Rutilus rutilus*" after the increase in the diameter of hydrated eggs, the hydrated eggs ratio of yolk sac size to the space around it increases but the hydrated eggs ratio of surface to volume decreases. In the under study "*Rutilus rutilus*" fishes the mean of the diameter of hydrated eggs (millimeter) was 2.28±0.10 and the mean of the diameter of yolk sac (millimeter) was 1.17±0.05. After increase in diameters of hydrated eggs and yolk sac, the ratio of surface to volume increased. In all fishes (including "*Rutilus rutilus*") after the increase in the diameter of hydrated eggs, the surface and the volume of hydrated eggs will increase; however, the surface increases more than the volume. Thus, after the increase in the diameter of hydrated eggs, the ratio of surface to volume will decrease [14, 11].

Regarding "*Rutilus rutilus*" phylogenic position including yolk sac size, the space around the yolk sac (Figure 1), the diameter of yolk sac (millimeter) and the ratio of hydrated eggs surface to its volume (Figure 2), it can be concluded that in this species yolk sac occupies more than half of hydrated eggs volume; and in comparing with other species (figure 1), "*Rutilus rutilus*", regarding the yolk sac size, comes after *Salmo trutta*, *Gasterosteus aculeatus*, *Esox lucius* and *Coregonus lavaretus* in which these species, ratio of yolk sac size to

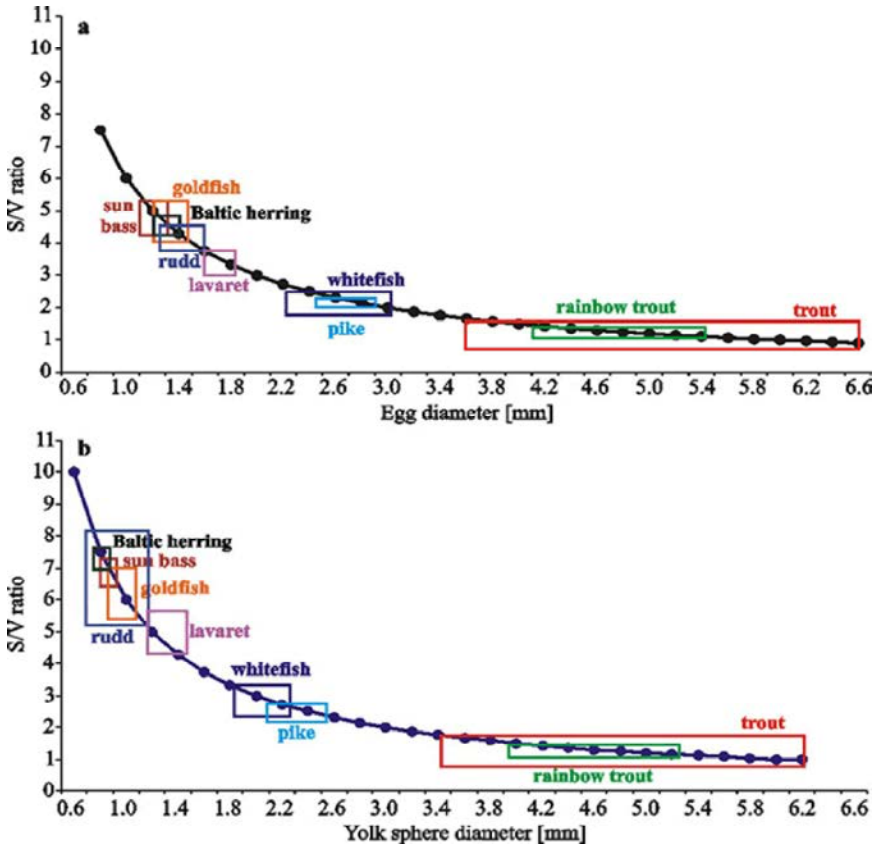

Fig. 2: Comparison of the ratio of surface to volume of hydrated eggs with diameter of hydrated eggs (millimeter, a) and diameter of yolk sac (millimeter, b) in some fish species and of "*Rutilus rutilus*" in occasion study

the space around it is more than "*Rutilus rutilus*" and it be concluded before *Coregonus albula*, *Perca fluviatilis* and *Carassius auratus* in which, above parameter is lower than in "*Rutilus rutilus*". In comparison with sun bass (*lepomis gibbosus*), in "*Rutilus rutilus*" the diameter of yolk sac decrease from 1.17 to 0.86 millimeters; accordingly, the ratio of yolk sac size to the space around it will decrease from 1.33 to 0.26. This means that although the diameter of yolk sac of "*Rutilus rutilus*" is only 1.36 times larger than that of sun bass, the ratio of the yolk sac to the space around it in "*Rutilus rutilus*" is 5.12 times more than that of sun bass (figure 1 and 2, [11]).

In comparison with sun bass, with the means of hydrated eggs diameter equal to 1.25 millimeter and hydrated eggs volume equal to 1.03 cubic millimeters, Trout, with the means of the hydrated eggs diameter equal to 5.01, has the hydrated eggs volume equal to 71.12. Therefore, there is a difference of 69 times between these two fishes, regarding the hydrated eggs volume, while the difference between their hydrated eggs diameters is only 4 times. Thus, the ratio of the hydrated eggs surface to its volume in Trout and sun bass is respectively 1.2 and 4.8 [11]. The same calculated ratio in "*Rutilus rutilus*" is 2.65. Another point to consider is the rate of vital structures of hydrated eggs. The reason that Trout hydrated eggs in cold water (6-10°C), "*Rutilus rutilus*" in water with an average temperature (15-18°C) and sun bass in warm water (25-27°C), can be described as following (figures and tables 1 and 2): the vital structure structures of hydrated eggs, in the process of embryo creation directly affected on metabolic rate; the ratio of spawn surface to its value in peripheral environment affects on the evaluation cycle of embryo [14]; the low ratio of surface to volume as well as high ratio of yolk sac size to the space around it, will increase the amount of needed oxygen [11] and this gas is transferred to vital structures of spawn. In warm water, Trout spawns, due to mentioned reason, will face some problems about oxygen and thus its spawns will perish (because of high metabolic rate and low oxygen in warm environment). Therefore, fishes in species evaluation process, through the environmental compatibility strategy will grow so that they hydrated eggs in environments containing enough oxygen; thus the embryo evaluation will occur in good situation [14, 15].

CONCLUSION

According what have been mentioned, researchers suggest that in all studies besides presenting hydrated eggs diameter some other factors such as yolk sac diameter, the ratio of surface to volume and the ratio of yolk sac size to the space around it should be affected. These factors are different between ovum and hydrated hydrated eggs and have a significant different between the diameter of ovum and hydrated eggs in different age classifications. The ratio of the surface to the volume in fertilized hydrated eggs was significantly lower than the same ratio in ovum, this ratio decreased after increase in the diameter of the ovum. The yolk sac space and the space around the yolk sac decreased after a decrease in the diameter of the hydrated eggs ($p<0.05$).

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