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Relationship Between Biological Characteristics of Egg with Fertility Success, Hatching Rate and Larvae Size in Female Kutum, *Rutilis frisii* Kutum

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Abstract: Since almost many published investigations on the properties and size of fish eggs were conducted without considering any other characteristics, such as the surface-to-volume ratio, yolk sphere and perivitelline space, therefore there is a gap to make a phylogeny correlation between species. Some biological characteristics of ovum (diameter, surface, volume and surface-to-volume ratio) as well as fertilized egg (including diameter, surface, volume, surface-to-volume ratio, yolk diameter, yolk sphere, perivitelline space and yolk sphere-to-perivitelline space ratio) were studies on fertilization success, hatching rate and larvae size among eighty female Kutum migrating to Tajan river. The range of ovum and fertilized eggs (hydrated) diameter were 1.9-2.44 and 2.41-2.87mm and the surface-to-volume ratio of ovum and fertilized eggs (hydrated) were 2.46-3.16 and 2.09-2.49mm^{-1}, respectively. There was a significantly direct positive relationship between ovum and yolk diameter ($p \leq 0.01$), yolk sphere-to- perivitelline space ratio ($p \leq 0.01$) and larvae size ($p \leq 0.05$) in second biometery. In contrast, relation between ovum diameter and surface-to-volume ratio ($p \leq 0.01$) and fertilized eggs surface-to-volume ratio ($p \leq 0.05$) was opposite. There was also significantly direct relationship ($p \leq 0.01$) between yolk diameter and yolk sphere-to- perivitelline space ratio as well as larvae size in second biometery. Direct relationship between hydrated egg and perivitelline space and opposite relationship between hydrated egg and surface-to-volume ratio ($p \leq 0.01$) existed. At first biometery there was significantly direct relationship between hatching success and larvae size ($p \leq 0.05$). In this species, larvae size increased with increasing the hatching success at first biometry, but at the second biometry no significantly relationship observed between these parameters. Regard to species evolution, during adaptation strategy to living environment, it spawns in a condition with enough oxygen in order to complete its embryogenesis successfully. Overall, it is suggested that in addition to the egg diameter in egg quality studies, yolk diameter, S/V and Ys/Ps ratio must be evaluated as well. It could help achieving a standard value for determining favorite or undesirable position of a species (with respect to S/V and Ys/Ps ratio) among the others.

Key words: Kutum *Rutilis frisii kutum* · Ovum · Eggs · Fertilization.

INTRODUCTION

Caspian kutum, *Rutilus frisii kutum* Kamenskii 1901, is an endemic fish of Caspian Sea and its populations generally recorded along near the coast, from the Trek River the north to the southern part [1]. It consists more than 70% of fishermen catch in Iran coastal of the Caspian Sea [2]. Kutum is a high market demand fish [3] and due to the dwindling of its natural resources by overfishing, dam construction in migration path and destruction of its natural reproduction environments, studies on this valuable species reproduction assist the aquaculture industry in meeting the ever increasing demand for kutum, by improving protocols for higher efficiency of egg production and enhanced survival of progeny [4].

Differences in egg dimensions have been correlated to spawning season [5, 6], the size of the individual fish [6-9], but also to brood protection [5, 9] absolute fecundity [10] and environmental factors [11], which are parameters that are especially difficult to detect in multiple batch spawners [5].

Almost many published investigations on the properties and size of fish eggs were conducted without considering any other characteristics, such as the surface-

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to-volume ratio, yolk sphere and perivitelline space and therefore makes us unable to make a phylogeny correlation between species [9].

The main objective of the present research was to determine whether any relationship between ovum (diameter, surface, volume and surface-to-volume ratio) as well as hydrated egg (including diameter, surface, volume, surface-to-volume ratio, yolk diameter, yolk sphere, perivitelline space and yolk sphere-to- perivitelline space ratio) can be established that may lead to a better determination of the phylogenetic position of *Rutilis frisii kutum* among other fish species.

MATERIAL AND METHODS

Broodstock Preparation: Our survey on possible correlation between a number of some biological characteristics of ovum (diameter, surface, volume and surface-to-volume ratio) as well as fertilized egg (including diameter, surface, volume, surface-to-volume ratio, yolk diameter, yolk sphere, perivitelline space and yolk sphere-to- perivitelline space ratio) with those female attributes such as length, weight, absolute fecundity and larvae size was carried out on 80 female migratory sexually mature kutum (*Rutilis frisii kutum*) with the body average weight of 977±229g (without the weight of gonad) and body length of 49.62±1.2Cm. For fertilization experiment 15 mature male with the weight of 735.7±241.7g and length of 44.2±4.9Cm was used. For establishing equal condition for fertilization and lowering the effects of male characteristics on the results, equal part of attaining sperm from all male speciments mixed [12]. After each female stripped separately, attaining ovum fertilized with mixed sperm and the fertilization success was determined in second cell fusion stage. Before fertilization, some ovum and hydrated eggs were fixed in acid acetic solution (5%).

Ovum and Hydrated Eggs Characteristic: Ovum and hydrated eggs evaluated under a loop equipped with ocular micrometer (with the accuracy of 100micro meter). For the measurement of egg diameter (mm), at first the largest and smallest diameter of eggs measured and then calculated the radius (r), diameter (d), surface (S) and volume (V) using the following formulas [13]:

$$d = (d1 + d2)/2,\ r = d/2,\ S = 4\Pi r^2,\ V = 4/3\Pi r^3$$ [6].

The surface-to-volume ratio, S/V, was also calculated. The volume (mm3) of the yolk space was calculated using the formula [9], Ps = V - Ys where Ps is the perivitelline space, V is the egg volume and Ys is the yolk space; the Ys/Ps ratio was calculated by dividing the yolk space by the perivitelline space.

For investigating the phylogenic position of *R. frisii kutum* eggs relative to other fish species, the biological characteristics identified in our study compared with those of other species previously published in the literature [9, 14]

Fertilization Success and Larvae Evaluation: For determination of fertilization success, due to the thick chorionic layer, fertilized eggs placed in acetic acid for 10 minutes. Then, under a loop equipped with ocoular micrometer (with the accuracy of 100 micro meter), the amounts of fertilized eggs and the stage of cell division determined.

At the end of the experiment, fertilized eggs transferred to the hatchery located in shahid Rajaee- Sari. Upon the hatching, hatching rate and larvae size was measured. In order to determine the hatching rate of each broodstock, the number of fertilized eggs which transferred to each incubator as well as the number of larvae from each incubator, belonging to each female, calculated using the following formula: number of egg (or larvae)= number of egg(or larvae) in gram × the weight of all attaining eggs in gram. Hatching success is calculated by dividing the number of larvae by the ovum number, according to the following formula: Hatching success= number of larvae / number of ovum×100.

To determine the larvae size, they immobilized with formalin solution (5%) and measured under oculometer loop and this operation continued until yolk sack disappeared.

Statistical Analysis: The correlation between biological characteristic of ovum and hydrated eggs and their relation to fertilization success, hatching rate and larvae size analyzed with Pearson correlation procedure. In addition, biological characteristic of ovum and hydrated eggs were compared using t-test (SPSS, ver. 10.05; SPSS, Chicago, IL).

RESULTS

Measures (mean±standard deviation) of a number of biological characteristics of *R. frisii kutum* ovum and hydrated eggs as well as some of the other species previously evaluated are presented in Tables 1 and 2.

Table 1: Biological characteristics of hydrated eggs and yolk spheres in several fish species

	Diameter(mm)		Volume, (mm3)		Surface area, S (mm2)		S/V ratio (mm-1)	
Species	Egg	Yolk sphere	Egg	Yolk sphere	Egg	Yolk sphere	Egg	Yolk sphere
Trout (Salmo trutta L.)	5.01±0.80	4.88±0.59	71.12±34.11	63.58±22.72	81.00±25.9	75.97±18.11	1.23±0.19	1.25±0.15
Rainbow trout (Oncorhynchus mykiss Walb.)	4.9±0.38	4.70±0.37	62.77±14.49	55.24±15.75	75.94±11.76	69.73±10.80	1.23±0.1	1.29±0.10
Pike (Esox lucius L.)	2.68±0.11	2.31±0.11	10.33±1.24	6.50±0.87	22.90±1.84	16.81±1.51	2.23±0.10	2.60±0.12
Lavaret (Coregonus lavaretus L.)	2.61±0.15	1.99±0.16	9.42±1.58	4.25±1.05	21.49±2.45	12.61±2.07	2.31±0.14	3.03±0.25
Whitefish (Coregonus albula L.)	1.78±0.10	1.34±0.12	3.00±0.50	1.29±0.35	10.04±1.10	5.68±1.03	3.37±0.18	4.52±0.42
Bream (Abramis brama L.)	1.64±0.04	0.99±0.04	2.33±0.18	0.51±0.06	8.51±0.45	3.09±0.24	3.65±0.10	6.07±0.25
Three-spined stickleback (Gasterosteus aculeatus L.)	1.52±0.06	1.33±0.33	1.83±0.22	1.24±0.15	7.23±0.57	5.57±0.44	3.96±0.16	4.51±0.17
Bleak (Alburnus alburnus L.)	1.48±0.06	0.96±0.04	1.71±0.22	0.46±0.06	6.90±0.59	2.90±0.25	4.06±0.18	6.27±0.28
Rudd (Scardinius erythrophthalmus L.)	1.33±0.07	0.88±0.11	1.26±0.21	0.38±0.14	5.62±0.61	2.5±0.63	4.50±0.23	6.88±0.85
Spring Baltic herring (Clupea harengus membras L.)	1.31±0.02	0.78±0.02	1.17±0.07	0.25±0.02	5.36±0.20	1.90±0.12	4.60±0.09	7.73±0.24
Perch (Perca fluviatilis L.)	1.28±0.04	1.00±0.05	1.10±0.11	0.52±0.08	5.15±0.33	3.12±0.31	4.69±0.16	6.04±0.31
Goldfish (Carassius auratus L.)	1.29±0.05	0.95±0.07	1.14±0.14	0.46±0.10	5.26±0.42	2.86±0.42	4.64±0.18	6.34±0.47
Sun bass (Leucaspius delineatus Heck.) 100	1.25±0.04	0.86±0.04	1.03±0.10	0.34±0.05	4.93±0.33	2.35±0.23	4.80±0.16	6.96±0.36

Data are presented as the mean±standard deviation

Table 2: Biological characteristics of Kutum ovum and hydrated eggs taken from brood stock captured in Tajan River, Iran

	diameter (mm)		surface (mm2)		volume (mm3)			
parametes	total	yolk	total	yolk	Total	yolk	S/V (mm-1)	Ys/Ps
ovum	2.14±0.14[a]	14.49±1.96[a]	5.22±1.1[a]	2.81±0.18[a]				
hydrated egg	2.61±0.12[b]	2.04±0.15	21.5±1.93[b]	13.08±1.9	9.4±1.27[b]	4.48±1.00	2.3±0.1[b]	1.00±0.46

Data are presented as the mean±standard deviation.

Table 3: Statistical Pearson's correlation between a number of the biological traits of the Kutum ovum and hydrated eggs

Variables	ovum diametr(mm)	hydrated eggs(mm)	Hatching rate (%)	Fertilization success (%)
yolk Diameter	0.988**	0.46	-0.277	0.104
perivitelline space	-0.247	0.682**	0.167	0.198
Ys/Ps	0.739**	-0.133	-0.307	0.01
S/V (ovum)	-0.997**	-0.558**	0.248	-0.176
S/V (hydrated eggs)	-0.545*	-0.998**	0.016	-0.307
Larvea size(First biometry)	0.055	0.372	0.595*	-0.239
Larvea size(second biometry)	0.614*	0.303	0.137	-0.154
fertilization success	0.185	0.313	-0.405	0.405
Hatching rate	-0.279	-0.036	0	-0.405

*P<0.05, **P<0.01 Figure 1. Relationship between the surface-to-volume (S/V) ratio (in mm-1) and yolk sphere diameter (a) and egg diameter (b) in several ?sh species, including the Kutum.

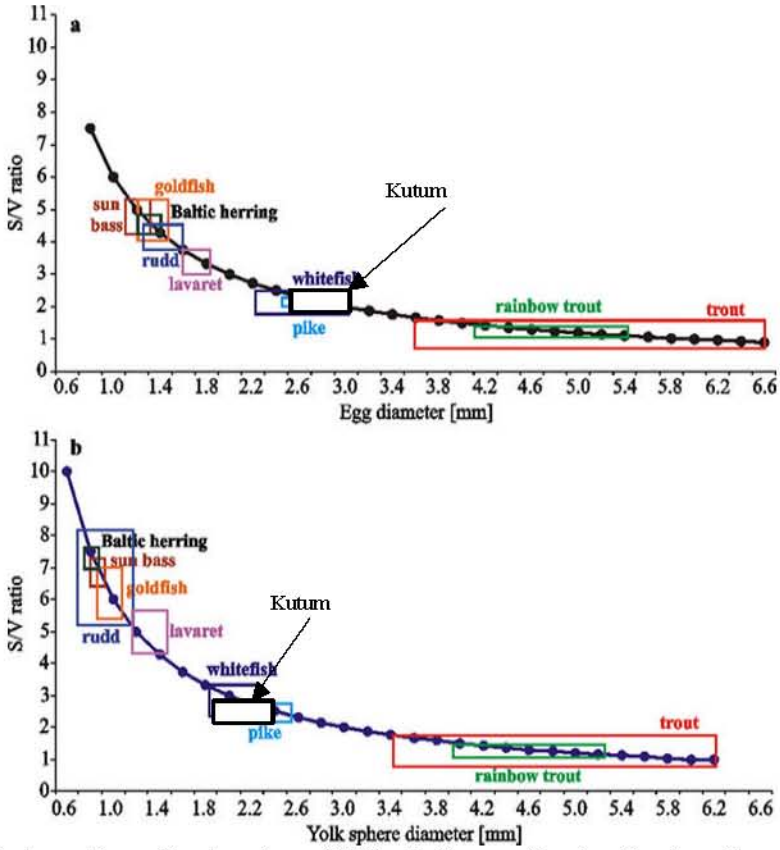

Fig. 1: Relationship between the surface-to-volume (S/V) ratio (in mm-1) and yolk sphere diameter (a) and egg diameter (b) in several ?sh species, including the Kutum

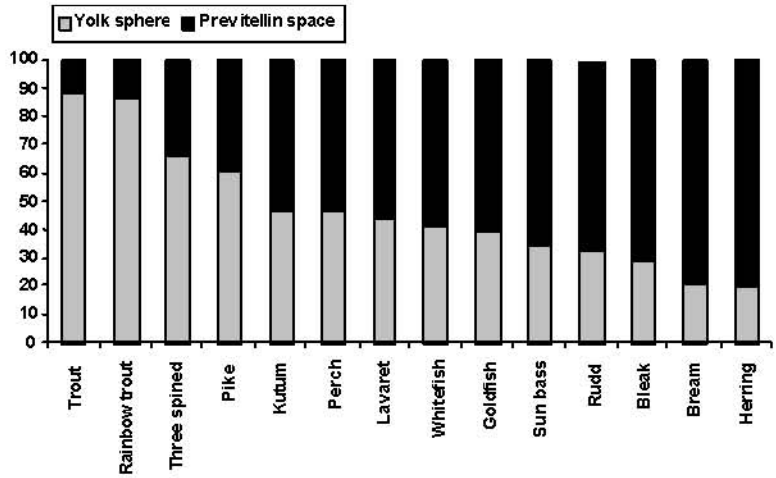

Fig. 2: Relative yolk space and perivitelline space (in %) inside hydrated eggs of different ?sh species, including the Kutum (from Imanpoor and Alavi, 2009).

The statistical c orrelation between a number of biological characteristics of the ovum and hydrated egg and hatching rate as well as fertilization success are presented in Table 3.

The phylogenic position of *R. frisii kutum* eggs relative to other fish species, with regard to the surface-to-volume ratio to the hydrated eggs and yolk diameter (millimeters) and yolk space-to- perivitelline space is presented in Figures 1 and 2.

DISCUSSION

The collected data referred to kutum showed that Yolk diameter, yolk sphere-to- perivitelline space ratio and larvae size (in second biometery) increased with increasing ovum diameter. On the contrary, surface-to-volume ratio of ovum and hydrated eggs decreased with increasing ovum diameter. In addition, hydrated eggs diameter increased, due to the hydration and perivitelline space formation [6].

Related to the correlation between ovum diameter as well as yolk with the larvae size, available results in literature showed increasing in these two parameters leads in to the increasing in larvae size. However, this correlation is observed just in the early life stage and is getting disappeared upon the larvae is growing, which is in accordance with previousfinding [15, 16].

There was no significantly relationship between fertilization success and the other parameters, as shown in table 3. It is possibly due to the high fertilization success in all broodstocks. If it was true, it could explain the non-direct correlation between quantitative biological traits (such as diameter of ovum, hydrated eggs, yolk and….) and eggs quality (fertilization success in this case). It is due to the relative variation among these quantitative biological traits, which lead to the no apparent difference fertilization success [15].

In Kutum hydrated eggs, the yolk space-to-perivitelline space ratio increases with the yolk sac diameter increasing. It means that the embryos (larvae) hatched from the eggs with the high yolk space, regard to the higher nutrition reserve, can withstand the hard conditions (with the exception to the low oxygen) as compared to their peer with the lower yolk space. How ever, the higher the yolk sac space the higher danger of low dissolved oxygen in their environment [16, 17]. Because For proceeding life processes correctly and for the energy stored in the yolk sac to be released, it is necessary that oxygen be available to the eggs and diffuse inside. In each species, this gas exchange area is predictably related to yolk sac as well as egg diameter and its volume. When embryo required high oxygen (because of the high yolk space to perivitelline space ratio), surface-to-volume (S/V) ratio, which is a value for determining the level of available oxygen to embryo, gets decreased and leads to mitigate oxygen diffusing from the ambient medium into an egg through the area corresponding to this interfacing surface.

In addition to the importance of the egg yolk volume, there is a higher relative contribution of the yolk sphere, (the living part of the egg) in large ?sh eggs; for example, this contribution is about 88% in the salmon egg [18, 19], decreased with decreasing egg size to values in the eggs of cyprinids (about 45% in *R. frisii kutum*)(Fig. 2)

This relationship constitutes a crucial element in the developmental strategy of a given species. Therefore, in nature, we usually observe fish with large, medium and small size eggs among psychrophilus, temperate and thermophilous ?sh, which are partly due to the factors discussed above concerning the S/V ratio, female gametes have been evolving towards a smaller size in low latitudes, result in the yolk sac size reduction.

Phylogenic position of Kutum with regard to the yolk space to privitelin space ratio and surface to volume ratio to the egg and yolk diameter (mm) showes that approximately the haif of the egg space is filled with yolk space and compare to the other species (Fig 2) it situated after trout, three stickleback, pike and before perch, Lavaret and whitefish related to the yolk space. Yolk diameter is 2.04mm in Kutum and decrease to 0.86 mm in the sunbass, consequently the yolk space to perivitelline space ratio decrease from 0.911 in Kutum to 0.26 in sunbass. It can be observed in figs.1 and 2 that despite the yolk diameter is getting 2.37 times larger than sunbass, yolk space to perivitelline space ratio is getting 3.5 times larger [9].

In kutum, like the other species, with increasing in egg diameter, the egg surface and volume increase, but volume increasing is higher than increasing surface which lead to decreased surface-to-volume ratio [6, 9].

Decreasing in S/V ratio accompanying with high yolk space to perivitelline space ratio, oxygen demanding is getting high, because of the high metabolic rate [9, 20]. Since oxygen is diffused into developing eggs, it can be concluded why Kutum is spawned in waters with medium temperature (Table 1, Fig.1 and 2). Consequently, in species evolution, during adaptation strategy to living environment, it spawns in a condition with enough oxygen in order to complete its embryogenesis successfully [6, 9].

Regarding to the above findings, it is suggested that in addition to the egg diameter in egg quality studies, yolk diameter, S/V and Ys/Ps ratio be evaluated as well. It could help to achieving a standard value for determining favorite or disaster position of a species (with respect to S/V and Ys/Ps ratio) among the others.

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