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The Relationship Between Egg Size, Fecundity and Fertilization Rate in *Acipenser persicus*, *Rutilus ferissi kutum* and *Cyprinus carpio*

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Abstract: The relationship between egg size, fecundity and fertilization rates were investigated in three fish of Caspian Sea. Sperm and eggs were collected with hypophysis treatment from *Acipenser persicus* and *Cyprinus carpio* L. and without hypophysis treatment by abdominal massage from *Rutilus ferissi kutum*. The male used for spawning was selected among those high quality of fish for three species. The spermatozoa motility was in average 85- 92. Statistical analysis shows a significant positive correlation for the relationship between fertilization rate and egg size. Fertilization rates was 71.5, 75 and 93 % and correlated positively with egg size ($r=0.9$, $P<0.05$; $r=0.09$, $P>0.05$; $r=0.565$, $P<0.05$) for *Rutilus ferissi kutum*, *Acipenser persicus* and *Cyprinus carpio*, respectively. There negative correlation was evaluated between fertilization rate and fecundity. We discuss the importance of producing variable sizes of eggs and speculate that this variability may be unpredic environment or part of a density-dependent regulatory mechanism and have effect on fertilization rate.

Key words: Fertilization rate · Fecundity · Egg size · *Acipenser persicus* · *Rutilus ferissi kutum* · *Cyprinus carpio*

INTRODUCTION

The development of oocytes in the gonad is not uniform, resulting in eggs variation in size and development.

During oocyte maturation, some morphological changes occur in the oocyte together with progression of meiosis. To equalize the development of eggs mostly it is necessary using hormones, e.g. to induce oocyte vitelogenesis and ovulation of *Mugil cephalus* and *Chalcarburnus chacoides,* the use of hormone is necessary [1, 2]. One of the functions of hormone is stimulation of the gonad to release the eggs. Therefore the eggs that is under hormonal treatment are release unequal size. In the other hand it seems in insufficient sperm concentrations, eggs with larger size had more chance of being fertilized.

Egg sizes vary extensively between and within species of marine invertebrates [3, 4]. The trade-off between fecundity and offspring survival was noted [5]. If eggs actually produced are not of minimal size, the larger size of the eggs must confer some survival advantage over a smaller size. Any factor that changes the relationship between egg size and probability of survival may then change optimal egg size [6]. There are several factors may have effect on egg size e.g. predation rate, food availability and water temperature [3]. It is also reported that differences in egg dimensions have been correlated to spawning season and the size of the individual fish [7, 8, 9], environmental factors [10], absolute fecundity [11] and biological characteristics of egg [12].

Un properly developed egg that are phenotypically smaller than the larger size take longer to develop during hatchery period consequently minimizes the number of survival offspring or in optimal egg size can be found relationship between egg size and survival probability. In stock assessment and population density to predict the available catch several factors are considered. Population density has been found to be a strong predictor of fertilization success [13] and has been used to explain differences among species in gamete traits such as sperm swimming speed, egg size and gamete longevity [14, 15].

Studies on the valuable species reproduction assist the aquaculture industry in inland rearing and restocking specially those endangered fishes such as sturgeon fish by improving protocols for higher efficiency of egg production and larval survival. The objective of present

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study was to evaluate the effect of egg size in fertilization which is important in aquaculture and restocking management.

MATERIALS AND METHODS

The biological data including egg size, fertilization rate and female attribute such as weight, length, absolute fecundity were survey and were carried out on each of 50 female migratory sexually mature Kutum (*Rutilus frisii kutum*), sturgeon fish (*Acipenser persicus*) and common carp (*Cyprinus carpio*) with the body average weight of 971±17g, 28.1±4.3kg and 1450±337g, respectively. This study was carried out at a local farm and the hatchery of Shahid Rajaei-Sari, during (2007-2009). For establishing equal condition for fertilization and lowering the effect of male characteristics on the results we used the semen of those fishes with high motility (85-92%) and based on the practical protocol of Shahid Rajaei fish propagation farm, sperm of different male specimens were mixed. The females of different treatments were stripped separately, attaining ovum fertilized with mixed sperm and the fertilization success was determined in second cell fusion stage. A part of ovum (approximately 1g) was separated and after weighting with digital balances. The eggs were counted to score the egg size. All fertilization trials were done as 3 replicates in sterile Petri dishes with 100±10 eggs. The dry fertilization methods were used and the insemination dosage was 1 percent of egg volume for each fertilization experiment. For fertilization sperm sample were poured onto the eggs and gently mixed about 20 second. Then 5 mL fertilization solution or clean fresh hatchery water was added and shacked gently. After 1 min the extra water of Petri dish were removed and the eggs were rinsed in hatchery water. Fertilization rate was determined as the percent of second cell fusion stage. For determining of fertilization success, the eggs were placed in acetic acid for 10 min then were checked by visual microscope.

Result is presented as mean ± standard deviation. Significant mean were subjected to a multiple comparison test (Duncan) for post-hoc comparison at α=0.05 level. All comparison and correlation test were carried out by SPSS, 17 for windows software package.

RESULTS

The spermatozoa motilities were in average (±SD) 92±4.3%, 85±10.2% and 85±6.7% for *Rutilus ferissi kutum, Cyprinus carpio* L and *Acipenser persicus,* respectively. The average and range of weight, length, fecundity and egg size are summarized in 1. The fertilization rates were 75±12 % (range: 45-96), 93±5 (range:75-98) and 71.5 ± 7.6 (range: 62-79) for *Acipenser persicus*, *Rutilus ferissi kutum* and *Cyprinus carpio*, respectively. In case of Kutum and common carp Statical analysis showed a significant positive allometry for the relationship between fertilization rate and egg size ($P<0.05$), besides a negative correlation between fertilization rates and fecundity were observed.

DISCUSSION

It is well known that the size of eggs of fish shows considerable intra- and inter- specific variation. Even personal fish of the same strain, weight and length have eggs that in different size [10]. Egg size is preliminary determined by the genotype of parental of fish and it is also known to be affected by other factors including age and size of the female parent. Alteration in egg size also occurs in batch-spawning fish as the season progresses [10]. Regardless of the origin or causes of variation (that we had not test in our study), there was significant difference between and within the eggs of the present study.

As stated earlier the eggs of a fish are varied in size and not uniform. In artificial propagation understanding these characteristics, we may synchronize the state of

Table 1: Mean weight, length, fecundity, egg size and fertilization of the *Rutilus ferissi kutum*, *Cyprinus carpio* and *Acipenser persicus*

Species	Value	Weight (g)	Length (cm)	Total fecundity (egg/fish) thousand	Egg size (No/g)	Fertilization rate (%)
Rutilus ferissi kutum	Mean±SD	97±17	39.7±.32	57±1.1	320±4	93±5
	Range	800-3380	30-56	22.9-107	216-434	75-98
Cyprinus carpio L.	Mean±SD	1450±33	42.3±2.9	114±50	743±92	71.5±7.6
	Range	940-2440	38-47	43.7-254.7	538-889	62-79
Acipenser persicus	mean±SD	28.1±4.3(kg)	169.8±11.5	246±73	53±6	75±12
	Range	19-37	138-200	123-420	40-69	45-96

Table 2: Correlation between egg size, fecundity, fertilization rate of the *Rutilus ferissi kutum*, *Cyprinus carpio* and *Acipenser persicus*

Fish species	Parameter	Length	Egg size	Fecundity
Rutilus ferissi kutum	Egg size	0.562**		
	Fecundity	0.806*	-0.386**	
	Fertilizationrate	0.275*	0.772*	-0.380
Acipenser persicus	Egg size	0.09		
	Fecundity	0.528**	-0.016	
	Fertilizationrate	0.07	0.188	-0.116
Cyprinus carpio	Egg size	0.859**		
	Fecundity	0.874**	-0.886**	
	Fertilizationrate	0.450	0.451*	-0.359

* P<0.05 ** P<0.01

eggs and ready to ovulation. Several methods are used e.g. A double treatment with CPE was used in sturgeon fish farm to induce oocyte maturation and ovulation [16]. In propagation of sturgeon first we should be confirmed maturity of fish. Gonad maturity is detected by taking sample, using a plastic cylindrical probe. Before treatment the eggs are at different stage of maturity High degree of migration of germinal vesicle (G.V.) expressed by polarization Index (PI) indicated spawnable females. Gray mullet and *Chalcarburnus chacoides* eggs production and maturation in the gonad are gradual until a synchronous spawning and oocytes remain very variable in size [2]. In marine fish such as mullet the eggs are released in several step, a proportion of oocytes that are released are relatively a part of maturated of the oocytes in the gonad. Actually at final stage of maturation the size of eggs are changed due to stage of progress in meiosis action, lipid droplet coalescence and yolk globule coalescence which result in the clarification of the oocyte's cytoplasm, migration of the nucleus to the periphery of the oocyte and dissolution of the nucleus membrane and the volume of eggs increase due to water uptake [1, 16]. The females rarely released all of their eggs and it is likely that only the largest and ripest oocytes are released during a single spawning and the typical bimodal oocyte size frequency distribution present in a mature gonad becomes unimodal with a greatly reduced mean size [17, 18].

In the present study, based on Table 2, there was highly significant correlation between the length of fish and fecundity in all three fish were investigated. Indeed by increasing the weight or length of fish it is supposed the fecundity will be increased, however it is expected that heavier female produce larger eggs that it is in agreement with Gall [19] who have shown in studies of hatchery-reared trout that older and heavier females produce larger eggs than younger and smaller fish. The availability of food also affects egg size [20]. Conover and Munch, [21] also reported the same results that the size of the mother positively correlates with the size of eggs produced in fish. Egg size in invertebrates was shown to significantly affect the later performance and survival of post-larvae: Larger eggs usually produce larger individuals better able to compete with others [22] and have a higher probability of being fertilised at lower sperm concentrations [23, 24]. On the other hand, producing smaller eggs requires less energy and allows the production of more offspring. In evolutionary terms, the balance between the number and size of eggs in the clutch is reached when the greatest number of offspring becomes mature and participates in reproduction [25].

Based on the results obtained in the present study we find correlation between the egg size and fecundity in all three kind of fish. The correlation for *Rutilus ferissi kutum* and *Cyprinus carpio* L was significant. The correlation was negative indicate by increasing the number of eggs the size will decrease. There was minimum correlation between egg size and fecundity in *Acipenser persicus*, this is because we took the sample at the time of spawning of this fish. At proper time of spawning by applying hormone therapy the egg size will be equal, therefore no correlation is appear between egg size and fecundity in this fish.

There are correlation between egg size, egg number and survival probability of offspring and a selection pressure exerted by sperm limitation during external fertilization. Although larger eggs are indeed a larger target for sperm, producing larger eggs also implies making fewer of them [6].

In the present study we used three types of brackish water fish each of different type of treatment for their artificial reproduction. The eggs of sturgeon was collected at spawning time therefore there was very little variance between the eggs, in this respect very little correlation

observed between egg size and fecundity and fertilization rate. The Kutum are spawned after the fish migrate to the river and the eggs are extracted without any treatment, therefore high variable are exist in batch of the reproduced eggs. The differences in egg size as mentioned earlier make the higher chance for those bigger once to be fertilized. This is in agreement with those stated by Styan [26] that in theory; larger eggs require lower sperm concentrations to be fertilized successfully. In case of Common carp, the fish was collected at sea shore of Caspian Sea, therefore such as Kutum, high variable are exist in batch of the reproduced eggs.

Egg size was correlated highly with fecundity and fertilization rate in case of Kutum and Common carp. Levitan stated this mater such a way that maximum egg diameter was found to affect fertilization in echinoderms [23] because they offer a larger target to sperm. The vitelline envelope contains specific chemical receptors which are the site for the sperm to bind to the egg [27], larger eggs are more likely to be contacted by sperm. Despite this, it appeared that only larger cytoplasm diameter offered more sites for the sperm to enter the cytoplasm and fertilize the egg successfully.

The eggs size of each individual fish is may be affected by its genotype reproductive status, environmental conditions and geographical location of the female. In natural reproduction the fish is under effect of one or all of these parameters, therefore in fertilization of the eggs all of this parameter should be consider.

CONCLUSION

We conclude that egg size is relatively correlated with fecundity and fertilization rate. It is suggest before spawning of each type of fish the status of eggs should be studied, because differences in egg size may give poor result in fertilization rate.

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