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An Investigation on the Micropyle Number in the Ova of the Sturgeon Species in Caspian Sea

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Abstract: The micropyle number in the ova of sturgeon species from the South Caspian Sea was investigated. The study was conducted on female broodfishes of three species of sturgeon (Persian sturgeon *Acipenser persicus*, Stelates sturgeon *A. stellatus* and great sturgeon *Huso huso*) included 44 Persian sturgeon, 13 stellate sturgeon and 8 great sturgeon specimens. Fifty eggs were randomly collected from each broodfish and the micropyle number of totally 3250 eggs was determined. Out of 44 Persian sturgeon used, 14 specimens were collected from the south-east Caspian region (Golestan Province, Shahid Marjani Center) and 30 were from the south-west Caspian region (Guilan Province, Shahid Dr. Beheshti Center). The stellate sturgeon specimens were taken from the south-west and the great sturgeon specimens from the south-east Caspian region. The obtained result showed that the mean micropyle number in Persian sturgeon caught from the south-east Caspian region was 8, whereas in those caught from the south-west region was 9. There was no significant difference ($P>0.95$) in the micropyle number of Persian sturgeon collected from the south-east and south -west regions. The mean micropyle number determined for stellate and great sturgeon was 5. The outer diameter of the micropyle in the Persian, stellate, and great sturgeon were 22 μm, 21 μm and 22 μm and the inner diameters were 19 μm, 17 μm and 19 μm respectively.

KEY WORDS: Micropyle, Oocyte, Sturgeons, South Caspian Sea

Introduction

The Caspian Sea is well known in the world for its vast surface area, being the most important natural habitat of 6 species of sturgeons and for producing of 90 % the world's caviar. The presence of sturgeons in the Caspian Sea has also made it distinct from the other seas in respect to fishing and fisheries. The Caspian Sea is considered the main reservoir of these species in the world.

Sturgeon attain sexual maturity very late (in males after 8-12 years and in females 14-18 years), when they migrate to rivers for spawning.

There are small pore on the egg surface in the animal pole region called micropyle through which spermatozoa can penetrate the eggs. Sturgeon eggs are encased by a single or many layered leather like protein membrane which originates from specific cells. This membrane or chorion protects the unfertilized egg and the developing embryo. In between these layers, the micropyles are formed on the egg surface (micropyles can be seen in stage IV and rarely in stage III of sexual maturity). Micropyles are formed from specialized cells, in the animal pole on the outer surface of the egg. When the nuclear membrane disappears, the egg is completely matured, the micropyle emerges from the first polar body and it is distinctly visible (Ebrahimi Darche, 1994).

In most fishes a single micropyle has been reported in each egg, but in sturgeons several micropyles have been observed. In sterlet sturgeon 5-13 and in stellate 1-13 micropyles have been reported (Dettlaff & Ginsburg, 1993). However in another investigation Podushka (1993) reported 0-10 micropyles in stellate sturgeon. In the sturgeons, the presence of a large number of micropyles and high density of sperms results in polyspermi in eggs which usually die during embryonic development. Although there are a large number of micropyles in sterlet and stellate sturgeon, we still do not know whether the number of micropyles in the sturgeons found in the South Caspian Sea are equal in number to those the North Caspian. The present study attempts to determine the micropyle number in three species of sturgeon (Persian sturgeon, stellate sturgeon and great sturgeon) ova from the southern region of the Caspian Sea.

Materials and Methods

Eggs of the three sturgeon species (Persian, stellate and great sturgeon) were collected from the catch stations at the southern regions of the Caspian Sea (Shahid Beheshti Center, Guilan and Shahid Marjani Center, Golestan) (Table 1). Twenty grams of eggs (prior to fertilization) were collected from each fish, fixed in 4 % formalin and analysed for micropyle numbers in the department of physiology and biochemistry, International Sturgeon Research Institute Rasht, Iran.

Table 1. Broodfishes collected at the Shahid Dr. Beheshti and Shahid Marjani Centers

Date	Species	No. of fish	For propagation	Not for propagation	Caught at		Center
					Sea	Sefidrud river	
3/3/97	*H. huso*	2	2	----	2	---	Marjani
15/3/97	*A. persicus*	4	---	4	4	---	"
16/3/97	"	9	8	1	9	---	"
18/3/97	*H. huso*	4	4	---	4	---	"
24/3/97	"	2	2	---	2	---	"
6/4/98	*A. persicus*	3	3	---	3	---	Beheshti
9/4/98	"	2	2	---	1	1	"
11/4/98	"	3	---	3	2	1	"
15/4/98	"	7	7	---	6	1	"
15/4/98	*A. stellatus*	1	1	---	1	---	"
184/98	*A. persicus*	10	8	2	4	6	"
26/4/98	*A. stellatus*	4	4	---	4	---	"
29/4/98	"	3	1	2	3	---	"
3/5/98	*A. persicus*	5	5	---	4	1	"
3/5/98	*A. stellatus*	2	1	1	2	---	"
4/5/98	"	3	2	1	3	---	"

Fifty eggs from each broodfish were analysed. After the eggs were washed with distilled water, Then each egg was held between the thumb and forefinger so that the ring distinguishing the vegetal and animal pole was visible with the naked eye by using a blade the section was made to separate the animal pole from the vegetal pole. The part of the egg containing the animal pole (micropyles are present in this region) was taken and its contents removed by a needle. The inner part of the egg membrane was washed thoroughly with water to remove any remaining contents and prepared for examination under the stereo microscope.

The egg membrane was placed on a watch glass under the stereo microscope with the concave surface facing downwards. A drop of water was added to prevent

dehydration of the membrane and to aid in focusing the light from the stereo microscope. The membrane was examined under a magnification of 32-50 and the micropyle numbers were determined. The micropyle pore transmit light which is reflected on the optical lens.

Out of the 3250 eggs totally examined, 400 eggs belonged to great sturgeon broodfish, 650 to Persian sturgeon broodfish collected at the Shahid Marjani Center, 650 eggs stellate sturgeon broodfish and 1550 eggs to Persian sturgeon broodfish collected at the Shahid Beheshti Center. From the eggs collected of those broodfish not ready for artificial propagation, follicular membrane was removed at the time of egg preparing. Apart from counting the micropyle number, the diameter of the micropyle of some eggs was also measured using a light microscope.

The data obtained was processed by statistical programs, Quattro pro, Stat Graphics and T-test at confidence interval of 95 %.

Results

The obtained results show that for the 650 eggs collected from Persian sturgeon broodfish of south-east Caspian region, the micropyle number ranged from 2-16 with a mean number of 7.71±1.59 and for the 400 eggs collected from great sturgeon broodfish also belonging to the same region, the micropyle number ranged from 2-12 with a mean number of 5.19±1.59. For the 1550 eggs collected from the Persian sturgeon broodfish caught in the south-west region, the micropyle number ranged from 2-27 with a mean number of 8.74±1.74 whereas for the 650 eggs collected from stellate sturgeon broodfish of this region, the micropyle number ranged from 2-13 with a mean number of 4.77±1.5 (Figs. 1-4). Comparisons on micropyle number in the broodfishes is shown in Fig. 5.

Statistical analysis and comparative study conducted on micropyle number in the eggs of Persian sturgeon collected from the south-east and south-west of the Caspian Sea showed no significant difference ($P>0.95$).

The outer and inner mean diameter of micropyle opening of three examined species (*A. persicus*, *A. stellatus* and *Huso huso*) in the east and west part of southern Caspian region is shown in Table 2.

Fig. 1: A view of micropyles (4) in stellate sturgeon (*A. stellatus*) eggs caught in south-west Caspian region (x 20)

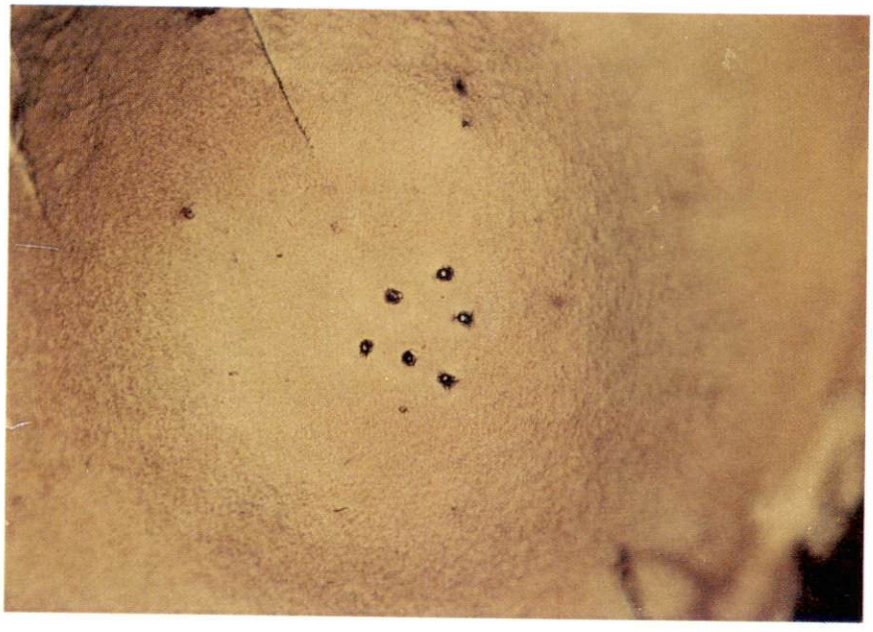


Fig. 2. A view of micropyles (6) in great sturgeon (*Huso huso*) eggs caught in south-east Caspian region (x 20)

Fig. 3. A view of micropyles (21) in Persian sturgeon (*A. persicus*) eggs caught in south-west Caspian region (x 50)

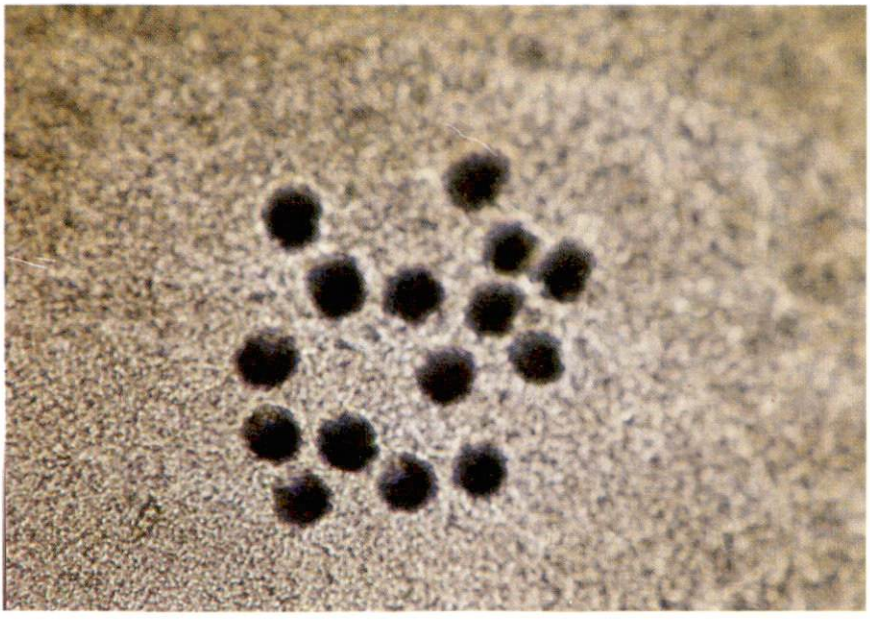
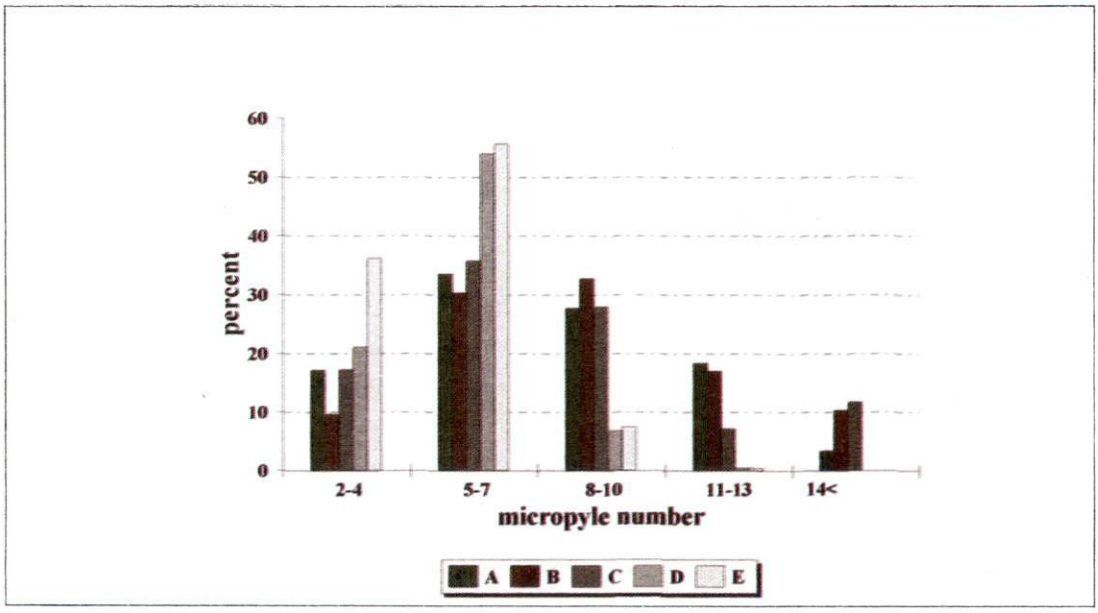


Fig. 4. A view of micropyles (15) in Persian sturgeon (*A. persicus*) eggs caught in south-east Caspian region (x 50)



A : Persian sturgeon (*Acipenser persicus*), Shahid Marjani Center
B : Persian sturgeon (*A. persicus*) from Shahid Beheshti caught in the Caspian Sea
C : Persian sturgeon (*A. persicus*) from Shahid Beheshti caught in the Sefidrud River
D : Stellate sturgeon (*A. stellatus*)
E : Great sturgeon (*Huso huso*)

Fig. 5: Comparison on the micropyle numbers of sturgeon fish from south Caspian Sea

Table 2. Micropyle diameter in sturgeons from the southern Caspian sea

Species	Inner membrane (μm)			Outer membrane (μm)		
	Min.	Max.	Mean	Min.	Max.	Mean
Acipenser persicus (east)	17.5	20.3	18.9±0.81	20.5	23.75	22.4±0.0.74
(west)	17.1	20.0	18.57±1.1	20.7	23.75	22.1±0.9
Acipenser stellatus	13.75	20.25	17.4±1.6	17.8	22.5	21.5±1.2
Huso huso	16.25	22.0	18.6±1.7	20.8	23.5	22.1±0.7

Discussion

In fish eggs the micropyle is the only opening through which the spermatozoa enters the egg and on fusion of the gametes in fertilization the zygote is produced.

The micropyle is funnel shaped leading to a canal. The micropylar canal forms an extension, ampulla from which a narrow terminal tubule extends. The micropyles are restricted to a small area near the animal pole. In stellate, great and Russian sturgeon, the distance between the outer openings of the micropyles is

usually 40-80 μm, and sometimes 100μm Micropyles are usually found in a specific part on the surface at the animal pole. In Russian sturgeon (*A. guldenstaedtii*) with 30 or more micropyles, the diameter of this part is up to 1000-1100μm. The micropyle area of eggs is 0.014-0.03 mm^2 of the animal pole surface (Dettlaff & Ginsburg , 1993).

The micropylar canal is short in the form of a tubule in the radiata zone. The number of micropyles is markedly different in various sturgeon species and also in eggs of one female. For example it varies from 1-13 in stellate sturgeon to 33 in great sturgeon and up to 52 in the Russian sturgeon (Dettlaff & Ginsburg, 1993).

The diameter of the micropyle pore is 13-17 μm and 6-7 μm at its narrow part (Kohne Shahri and Azari Takami, 1974). The diameter of the narrow tubule of the micropyle in each species is slightly more than the width of the spermatozoan head of the same species, a point that is taken into view in hybridization. Therefore the shape and dimensions of the micropyle differs from species to species and is sometimes used as a key to identification.

According to Podushka (1993) the micropyle number in Russian sturgeon is 0-45 with a mean number of 7.9 and in stellate sturgeon 0-10 with a mean number of 5.1±0.04 (Table 3).

In artificial propagation, the sperm collected from a male fish is diluted with fresh water several times more than its volume. Usually for every kilogram of eggs, 10 cc of sperm is added for artificial propagation. Dilution of sperm solution is carried out either due to the high density of sperm or the presence of a large number of micropyles in sturgeon eggs (Kohne Shahri and Azari Takami, 1974).

So far no studies have been conducted on the micropyle number of different sturgeon species in Iran, this is the first report in this respect. Out of 3250 eggs which were examined in this study, 650 eggs of Persian sturgeon collected from the Shahid Marjani Center, 17.07 % of the eggs bore 2-10 micropyles, 33.53 % had 5-10 micropyles, 27.69 % had 8-10 micropyles, 18.3 % had 11-13 micropyles and 3.3 % of the eggs were shown to 14 or more micropyles in each egg. On examination of 1050 eggs collected from Persian sturgeon broodfish (caught at sea) collected from the Shahid Beheshti center, 9.61 % of the eggs possessed 2-4 micropyles, 30.28 %, 5-7 micropyles, 32.76 %, 8-10 micropyes, 16.95 %, 11-13 micropyles and 10.38 % of the eggs possessed 14 or more micropyles in each egg.

The results of the 500 eggs taken from the Persian sturgeon (caught in the Sefidrud River) also collected from the Shahid Beheshti Center, 17.2 % of the

Table 3 : The micropyle numbers studied in some sturgeon species

No.	Species	Scientific Name	Micropyle No.	Mean Number	Reference
1	Stellate Sturgeon	*Acipenser stellatus*	1-13	less than 5	Kohne Shahri, Azari Takami, 1974 ; Dettlaff & Ginsburg , 1993
2	Stellate Sturgeon	*Acipenser stellatus*	0-10	5.1	Podushka , 1993
3	Stellate Sturgeon	*Acipenser stellatus*	2-13	4.77	Hallajiyan , 1998
4	Great Sturgeon	*Huso huso*	1-33	more than 25	Kohne Shahri, Azari Takami, 1974
5	Great Sturgeon	*Huso huso*	1-17		Kohne Shahri, Azari Takami, 1974 ; Dettlaff & Ginsburg , 1993
6	Great Sturgeon	*Huso huso*	2-12	5.19	Hallajiyan , 1998
7	Russian sturgeon	*Acipenser gueldenstaedtii*	1-52	more than 25	Kohne Shahri, Azari Takami, 1974
8	Russian sturgeon	*Acipenser gueldenstaedtii*	0-45	7.9	Podushka , 1993
9	Russian sturgeon	*Acipenser gueldenstaedtii*	1-43		Kohne Shahri, Azari Takami, 1974 ; Dettlaff & Ginsburg , 1993
10	Persian sturgeon	*Acipenser persicus*	2-27	8.24	Hallajiyan , 1998
11	Ship sturgeon	*Acipenser nudiventris*	5-13		Dettlaf & Ginsburg , 1993
12	German sturgeon	*Acipenser sturio*	3-9		Dettlaf & Ginsburg , 1993
13	White sturgeon	*Acipenser transmontanus*	3-15		Dettlaf & Ginsburg , 1993
14	Sterlet sturgeon	*Acipenser ruthenus*	5-13		Kohne Shahri, Azari Takami, 1974 ; Dettlaff & Ginsburg , 1993

eggs showed 2-4 micropyles, 43 % had 5-7 micropyles, 29.4 % showed 8-10 micropyles, 7.2 % showed 11-13 micropyles and 11.8 % of the eggs had 14 or more micropyles each.

In stellate sturgeon broodfish, the results of the 650 examined eggs showed that 38.92 % of the eggs had 2-4 micropyles, 54 % had 5-7 micropyles, 6.77 % had 8-10 micropyles and 0.3 % of the eggs had 11-13 micropyles in each egg.

The examination of 400 eggs taken from great sturgeon broodfish showed that 36.25 % of the eggs had 2-4 micropyles, 55.75 % had 5-7 micropyles, 7.5 % had 8-10 micropyles and 0.5 % of the eggs had 11-13 micropyles in each one. The micropyle number in different sturgeon species found in the South Caspian Sea is shown in Graph 1. As it is showed in this graph, the highest frequency of micropyle number in great sturgeon and stellate sturgeon was 2-7, and the lowest frequency was 7 or more in each egg. In these two species eggs having a micropyle number of 14 or more were not observed. However in contrast, in Persian sturgeon the lowest frequency of micropyle number was 7 or less and the highest frequency of micropyle number observed was 7 or more in each egg. On the whole, the highest micropyle number 27 was observed in Persian sturgeon broodfish caught in the Sefidrud River and the lowest micropyle number 12 was seen in great sturgeon.

The obtained results show no difference in micropyle numbers of stellate sturgeon broodfish belongs to the South Caspian and those to the North Caspian Sea, whereas in great sturgeon, differences in micropyle numbers were observed. The maximum number of observed micropyle in great sturgeon specimens belonging to the North Caspian Sea and was 33, whereas for those from the South Caspian Sea was 12.

Study on the causes of polyspermy and the way to prevent this phenomenon is interesting topic of research , where apart from determining the micropyle number we can also study the quality of eggs and dilution and quality of sperm.

Due to the presence of several micropyles, more than one spermatozoa can penetrate the egg simultaneously and cause polyspermy. Although the presence of several micropyles increases the incidence of polyspermy, the shortening of the micropylar canal to half its size after the penetration of the first spermatozoa, decreases the passage and restricts more sperm entry to the perivitelline space. At present, it is not known whether the mechanism to prevent polyspermy in sturgeon eggs exists or not. The poor quality of eggs may be the reason for the increase in

the percentage of polyspermic eggs. The spermatozoa can enter the cytoplasm of the eggs which are in stage 3, 4 of the development and the fertilized eggs can develop until the blastomere stage, which then will die in the further stages of development. In other words, most of the polyspermic eggs lead to the abnormal development of the embryo and their subsequent death. Only a few of them develop fully as abnormal worms or with undeveloped heads (Dettlaff & Ginsburg, 1993).

In any case, the Persian sturgeon which are mostly found in the southern region of the Caspian Sea is a suitable species to conduct such studies. We can also study the structure of the micropyle by using an electron microscope. Although studies on population and subspecies are conducted on the basis of electronic view of micropyles of one species in different geographical locations, such studies can be recommended for the indigenous species of Iran and other commercial species of the Caspian Sea.

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