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A Study on the Effects of Stocking Density of Eggs and Larvae on the Survival and Frequency of Morphological Deformities in Persian Sturgeon, Great Sturgeon and Stellate Sturgeon

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Abstract: The effects of stocking density of eggs and larvae were studied related to the morphological deformities appearance, percentage of survival, and growth rate, in order to achieve the most suitable stocking density for eggs and larvae in incubators and rearing tanks. The egg stocking densities were 500, 700 and 900 g per incubator for great sturgeon, 500 and 1000g per incubator for Persian sturgeon and 400 and 425g per incubator for stellate sturgeon. Larvae were stocked at densities of 10000, 15000 and 20000 larvae per m3 for great sturgeon, 21660, 41004 and 69000 larvae per m^3 for Persian sturgeon and 15770, 23250, 57660 and 62250 larvae per $m^3$for stellate sturgeon.

The results indicate significant differences in percentage of survived in fertilized eggs ($P<0.05$). By transferring the larvae into the fibreglass tanks, growth and survival rate decreased, whereas mortality and incidence of deformities increased as stocking density increased particularly on the onset of exogenous feeding.

In the present study totally 20 types of deformities including bent spine, hunched back, hanging or looped tail, fin deformation, stunted body length and etc. were registered. The results showed that the morphological deformities frequency in dead and live fish were higher in high stocking densities comparing to the low stocking densities.

KEY WORDS: Great sturgeon, Persian sturgeon, Stellate sturgeon, Morphological Deformities, Stocking Density, Larval Survival

Introduction

Sturgeons are one of the most important and valuable fishes found in the north hemisphere. The Caspian Sea is the main habitat of these fishes that produces about 90 % of the world's caviar. Due to the high adaptability to various environmental conditions, sturgeons have succeeded to survive from the Cretaceous period (about 150-200 million years ago). The increase of water pollution as a result of destructive factors in the Caspian Sea, disturbance in its ecological stability, illegal fishing, loss of natural spawning beds, development of oil extraction activities in the Caspian Sea and etc., have threatened the lives of these fishes (Bahmani, 1998). The presence of fishes with morphological deformities, low percentage of survival, premature mortality and etc., in breeding hatcheries are a few of the most important limiting factors in the conservation and restoration of these endangered species.

Environmental factors like increase in stocking density, fluctuations in temperature and dissolved oxygen concentration, pH, sediment matters, heavy metals i.e. copper and lead, chemical compounds pesticides, fertilizers, hereditary and pathogenic factors, and unsuitable feeding conditions (Mohseni *et al.*, 1997) are among the several factors leading to the appearance of deformities. Various mechanical and environmental factors in fish hatcheries during the process of breeding and rearing result deformities in larvae that may sometimes even lead to death.

Increase in stocking density of eggs in incubators and that of larvae in rearing tanks before and after onset of exogenous feeding may cause adverse effects on the production quality and above all on the ecological state of these fishes, due to crowding and lack of space and other operational restrictions. Observing the principles of stocking and rearing in hatcheries particularly in the case of endangered species (great and ship sturgeon) calls for research and attention.

The first incidence of skeletal abnormality and change in shape of spine in sturgeons and their hybrids was reported in great sturgeon by the researchers of the zoology laboratory of St. Petersburg University, Russia in 1985 (Frank, 1992).

Scientific reports show that highest mortality and incidence cases of deformities in fishes belong to the early stages of ontogeny and embryogenesis. Usually incidence of deformities occur simultaneously with the division of blastomeres which cause bent body, individuals with two heads or two bodies, asymmetry of yolk sac with respect to the embryonic axis and hemorrhage (Mohseni, *et al.*, pathological factors (increase in stocking density, fluctuations in temperature and dissolved oxygen concentrations, pH, etc.) (Hosseini, 1997). In Iran similar studies were conducted on Persian sturgeon and stellate sturgeon in 1997 in the framework of a contract between Iran and Russia and on great sturgeon in 1998. The extent and type of deformities in eggs and larvae of the species mentioned were examined at various stocking densities and the best stocking density for artificial rearing conditions was determined (on the basis of decrease in morphological deformities and death of larvae, enhancing available feeding potentials, production of healthy fingerlings for restocking programs and increase in fishery output).

Materials and Methods

Samples of eggs and larvae were collected during the breeding seasons in 1996 and 1997 from the sturgeon hatcheries (Shahid Marjani and Shahid Beheshti) and the International Sturgeon Research Institute. All incubators and rearing tanks were disinfected with 0.5 g/lit $KMnO_4$ solution before being filled. Tanks were cleaned and dead larvae were counted and removed everyday. Length, weight and daily growth of larvae were also measured and recorded. Egg samples were fixed in 5% formalin and brought to the laboratory. The quality of eggs at different stages of development was determined according to Dettlaff *et al.*, (1969) at stage 5, 16 (gastrulation) and stage 35 (before hatching) using a stereomicroscope. Fertilized, damaged, parthenogenetic, polyspermic and unfertilized eggs were identified and counted.

On hatching, the larvae were collected using scoop nets and transferred with care to fiberglass tanks (2x2x0.53 m) and concrete tanks with a capacity of 1m^3. Stocking densities used were 10000, 15000 and 20000 larvae per fiberglass tank for great sturgeon, 21660, 41004 and 69000 larvae per m^3 for Persian sturgeon and 15770, 23250, 57660 and 62250 larvae per m^3 for stellate sturgeon in concrete tanks. Each trial was run in three replicates. Everyday about 66 larvae (dead and alive) were randomly selected from each trial and fixed in 5% formalin. This was done throughout the rearing period. The larvae were weighed (using a digital balance with 0.001g precision) and their length were measured and recorded. Larvae were fed with live food (artemia and daphnia) at a rate of 25% of their total biomass at every 3-4 hour intervals. Water temperature and dissolved oxygen concentrations were recorded daily. Statistical analysis of data was conducted using Kruskal-Wallis, SAS, SPSS, Stat Graph and Quatro Pro for Windows software.

Results

Survival percentage of embryo at stage 5 of cell division, gastrulation and prior to hatching is presented in Table1. This was determined on the basis of number of eggs during each stage.

Table 1: Comparison of live eggs (%) at different stages of embryonic development

Stocking density (g/incubator)	Species	Survival of embryo at stage 5 (%)	Survival at gastrulation (%)	Survival prior to hatching (%)
500	Great sturgeon	83.70	75.87	64.6
700	Great sturgeon	77.97	66.50	56.9
900	Great sturgeon	68.97	60.57	46.2
500	Persian sturgeon	95.30	95.00	83.5
1000	Persian sturgeon	91.60	87.30	75.0
400	Stellate sturgeon	84.30	84.00	75.0
425	Stellate sturgeon	81.20	80.40	75.0

Comparison of growth trends in eggs in different trials indicates that survival of eggs decreased significantly with increase in stocking density (Table 1). A significant difference was observed among the different trials ($p < 0.05$). Eggs during incubation showed significant increase in mortality (Table 2). This mortality can be attributed to stocking density as well as to the quality and quantity of sperms. The study of growth trends and mortality of eggs during blastulation showed that the percentage of parthenogenetic eggs in trials with higher density was at least 2% higher than that in trials with low density.

Table 2: Comparison of fertilized, unfertilized, polyspermic and parthenogenetic eggs in sturgeon species

Stocking density (g/incubator)	Species	Survival of embryo at stage 5 (%)	Unfertilized eggs (%)	Injured eggs (%)	Polyspermic eggs (%)	Parthenogenetic eggs (%)
500	*H. huso*	83.7	12.8	2.3	0.2	0.9
700	*H. huso*	77.9	15.8	2.5	1.87	1.9
900	*H. huso*	68.9	22.9	2.8	2.4	2.9
500	*A. persicus*	95	3.1	-	1.6	-
1000	*A. persicus*	92	4.3	-	4.1	-
400	*A. stellatus*	84.3	4.5	-	0.5	10.7
425	*A. stellatus*	81.2	15.9	-	0.7	2.2

The duration of embryonic development in Persian sturgeon is reported as 2140.8 t° at 22.3° C, 1468.8 t° at 20.4° C in stellate sturgeon and 2556.8 t° at 16.39° C in great sturgeon.

On hatching, larvae were transferred to fiberglass and concrete rearing tanks. The average mortality (Fig. 1) and growth of larvae studied (at onset and during exogenous feeding) indicate that before the onset of exogenous feeding, the stocking density was not a limiting factor for larval growth however after onset of exogenous feeding it played a vital role in the growth of larvae.

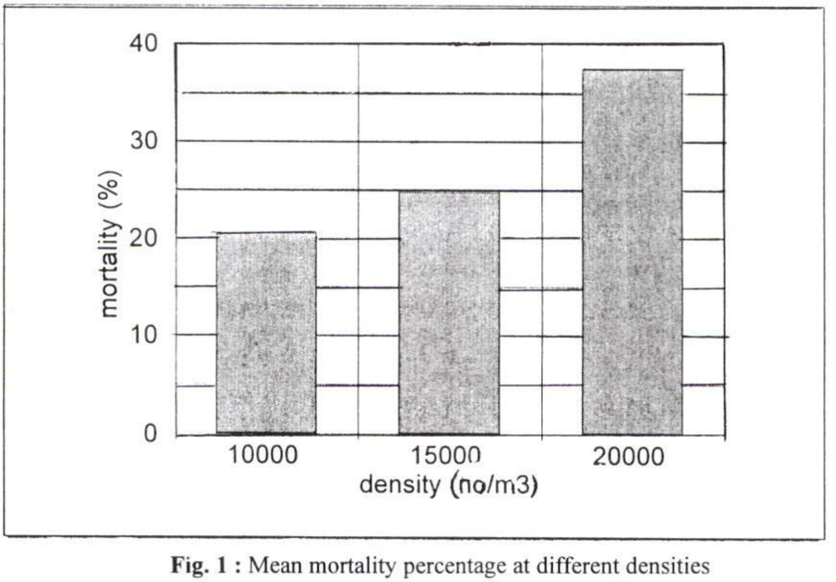

Fig. 1 : Mean mortality percentage at different densities

Morphological Deformities:

Study of the recorded deformities indicates that the frequency of deformities in trials with low stocking density was significantly different from that in trials with higher stocking density (Fig. 2-8).

Deformities were studied in dead and live larvae. Morphological deformities and signs of poor health were observed in dead as well as live larvae (Fig. 9-16). Twenty signs of morphological deformities including bent spine, hunched back, hanging or looped tail, deformation of left or right abdominal fins, stunted body length and etc., were observed in dead and live larvae (Table 3).

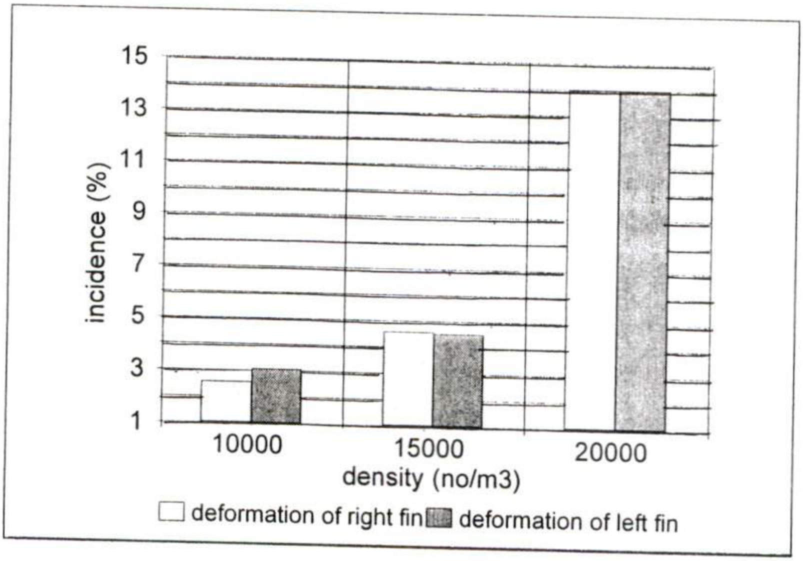

Fig. 2 : Comparison of incidence of morphological deformities (%) in dead fishes at different densities

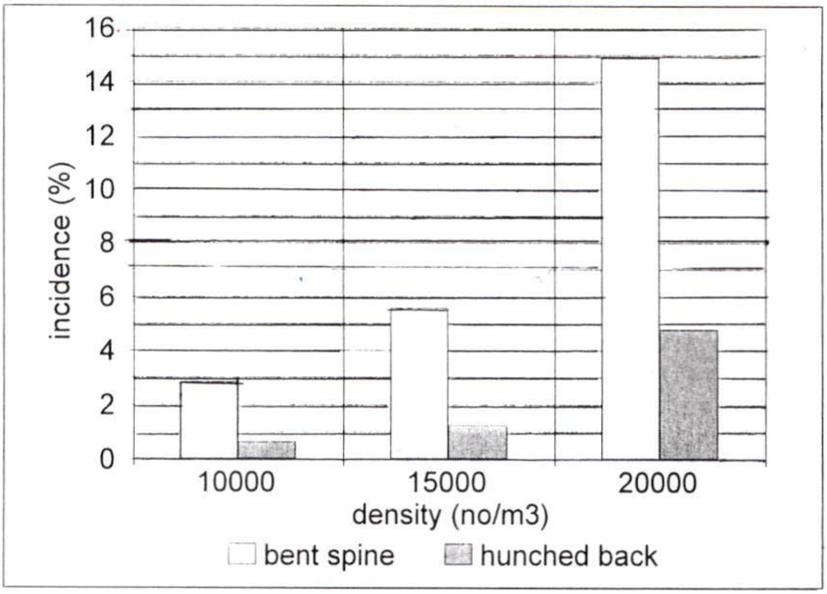

Fig. 3 : Comparison of incidence of morphological deformities (%) in dead fishes at different densities

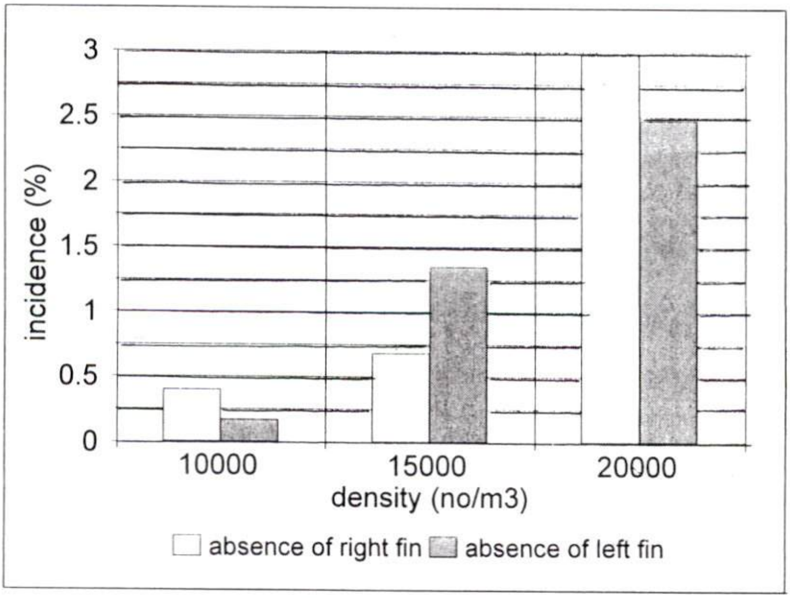

Fig. 4 : Comparison of incidence of morphological deformities (%) in dead fishes at different densities

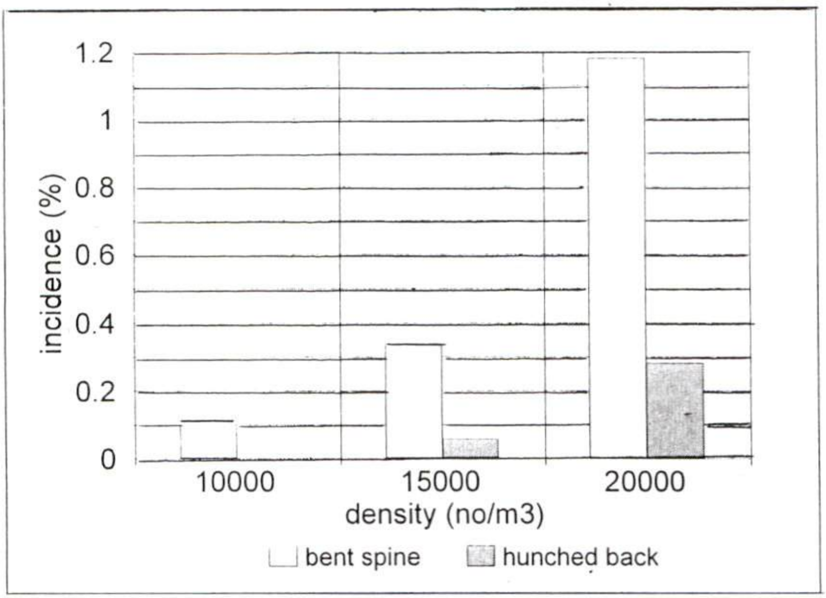

Fig. 5 : Comparison of incidence of morphological deformities (%) in live fishes at different densities

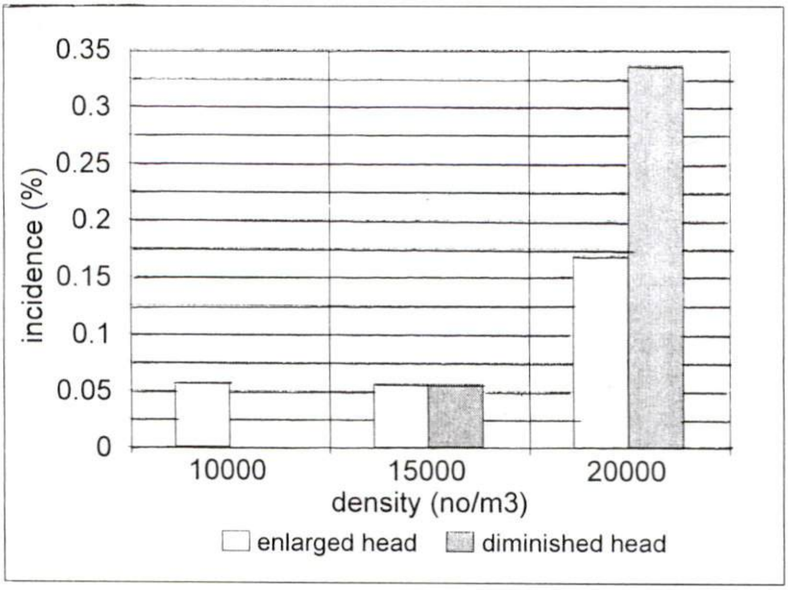

Fig. 6 : Comparison of incidence of morphological deformities (%) in live fishes at different densities

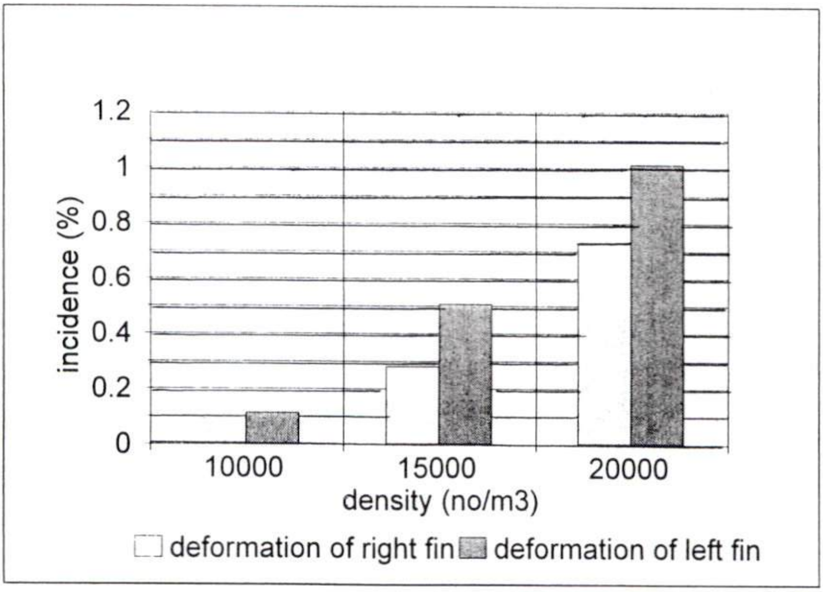

Fig. 7 : Comparison of incidence of morphological deformities (%) in live fishes at different densities

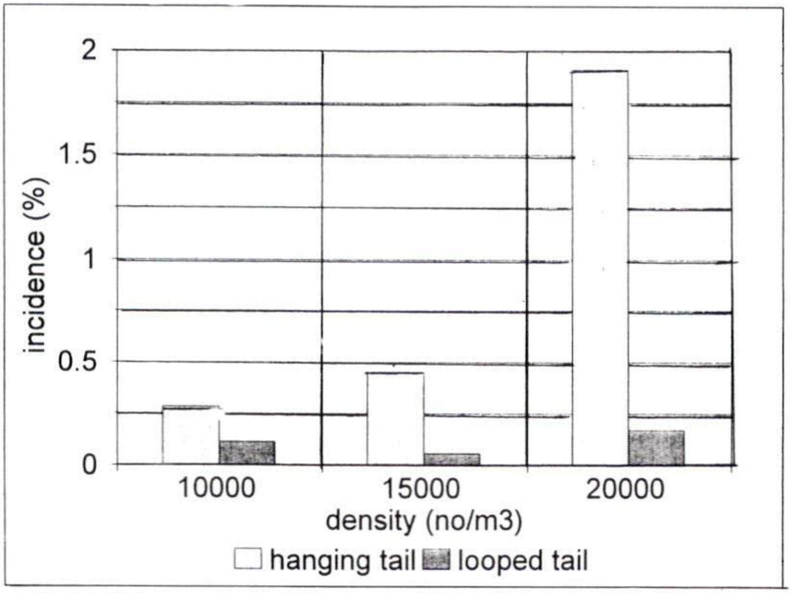

Fig. 8 : Comparison of incidence of morphological deformities (%) in live fishes at different densities

Fig. 9 : Larvae showing bent spine and deformed fin

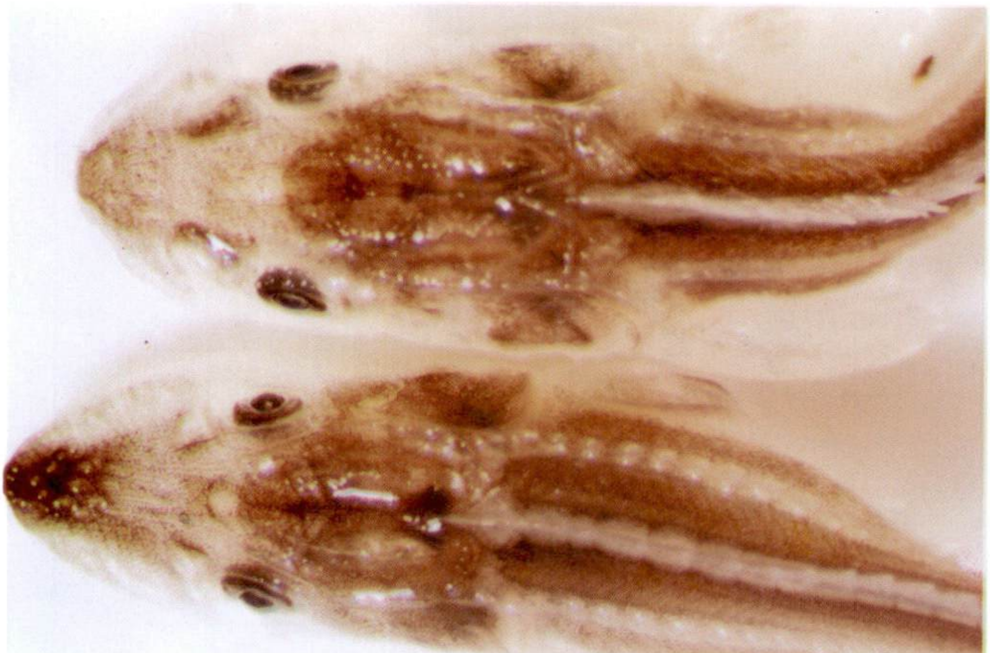

Fig. 10 : Larvae showing enlarged head

Fig. 11 : Larvae showing swollen yolk sac

Fig. 12 : Larvae showing hanging tail

Table 3 : Incidenses of morphological deformities in sturgeon species

	Mean incidence of deformities (%) in *H. huso* larvae						Mean incidence of deformities (%) in *A. persicus* larvae			Mean incidence of deformities (%) in *A. stellatus* larvae			
	in live fish			in dead fish									
	10000	15000	20000	10000	15000	20000	21660	41004	69000	15770	23250	57660	62250
Bent spine	0.11	0.34	1.18	2.81	5.56	14.9	2.12	6.06	7.27	0.61	1.82	6.66	6.05
Hunched back	0	0.06	0.28	0.62	1.23	4.78	1.52	6.06	2.18	0.91	0.06	3.33	1.82
Hanging tail	0.28	0.45	1.91	2.69	4.55	9.93	0.91	5.76	3.33	0	0	4.84	2.42
Looped tail	0.11	0.06	0.17	0.79	1.07	3.25	0.3	1.21	1.11	0.3	0	2.73	1.51
Deformed right fin	0	0.28	0.72	2.54	4.55	13.97	3.33	14	13.94	0	0	0	0
Deformed left fin	0.11	0.51	1.01	3.03	4.49	13.97	6.67	7.88	14.83	0	0	0	0
Stunted length	0	0	1.01	0	0	0.51	0	0	0	0.61	0	0	0
Deformed yolk sac	0	0	0.17	0.06	0.11	0.17	0	0	0	0	0	0	0
Condensed yolk sac	0	0	0.11	0	0.06	0.06	0	0	0	0.91	0.3	0.6	0.61
Swollen yolk sac	0	0	0	0	0.06	0	0	0	0	0.3	0	0	0
	22												
Right fin absent	0	0	0.34	0.39	0.67	2.97	0.3	0	0.3	0	0	0	0
Left fin absent	0	0	0.45	0.17	1.35	2.67	0	0	0	0	0	0	0
Enlarged head	0	0	0.06	0.06	0.06	0.17	0.3	0.3	1.2	0.3	0	0	0
Diminished head	0	0	0.11	0	0.06	0.33	0	0	0	0	0	0.3	0
Eyes absent	0	0	0	0.11	0	0.06	0	0	0	0	0	0	0
Barbells absent	0	0	0	0.34	0.56	1.79	0	0	0	0	0	0	0
Distended stomach	0	0.22	0.62	0.39	0.73	2.45	0.3	0	0.91	0.91	0	0	0
Neoplasmy	0	0	0	0	0	0	0	1.51	1.82	0	0	1.82	0
Cannibalism	0	0.06	0.45	0.34	0.56	1.97	0	0	0	0	0	0	0
Deformed head	0	0	0	0.17	0.51	0.89	0	0	0	0	0	0	0
Deformed body	0	0	0	0.62	1.12	2.69	0	0	0	0	0	0	0

Discussion

The distressed status of natural stocks of sturgeons and the loss of their natural habitats, over fishing, construction of dams and etc., have endangered the life of these species. On the basis of avaliable data, the natural spawning grounds for great sturgeon have been destroyed in most regions and the physiological state of this species has been reported to be more critical than that of stellate and Russian sturgeon (Pourkazemi, 1997). No data is available on the most suitable stocking density for sturgeon eggs and larvae in artificial rearing conditions. Also there is no evidence that an increase in stocking density could be a reason for mortality and morphological deformities. Results obtained from such researches show that embryonic development and production of healthy larvae depend on environmental conditions as well as the requirements of embryo during un-favorable conditions. It is evident from these findings that the use of water from a common source with similar water flow in incubators using different stocking densities of eggs results variation in the embryonic development of eggs and larvae. Overstocking of eggs leads to retardation in organogenesis, and increase in incidence of abnormalities, in percentage of abnormal larvae and mortality (Nazari, 1996). Although the number of abnormal juvenile fish has increased, research conducted showed that environmental factors such as mechanical injury and stocking density lead to impairment of embryogenesis and organogenesis (Chebanov, 1991).

On the basis of obtained results the average survival percentage of viable embryo in different stages (gastrulation, pre hatch) was higher in trials with lower stocking density comparing to that in the other two trials used (Table 1). Since all trials were run under similar environmental conditions, we may conclude that high mortality in trials with higher stocking density is a result of over crowding of eggs in the incubators. Embryos that lie in the center of this mass develop very slowly and showed different deformities and usually died before hatching. Another factor is the absence of vital gas exchanges (CO_2 and O_2) due to large number of embryo in each incubator. The occurrence of this phenomenon in natural environments support the obtained results from the present study. Lowest mortality in natural environments was reported about eggs stocked at low density in the riverbed during spawning (Nazari, 1996). Such results have been reported for salmon eggs under artificial conditions. Higher stocking density in incubators results in increased mortality (Clyde *et al.*, 1986). Also on the basis of investigations carried out, examination of eggs during synchronous division of blastomeres showed polyspermy, parthenogenesis and damaged eggs.

Studies conducted in natural environments have related these physiological abnormalities to pollution (Abtahyee, 1998). Since all physical and chemical factors of water used were maintained constant under experimental conditions in all incubators, we can state that the appearance of these deformities in high stocking densities is most probably the result of a large number of eggs per unit of surface in each incubator. Findings of McMenemy (1995) and Mohseni (1998) also support this view.

The results for great sturgeon larvae indicate that growth (weight and length) of fingerlings in trial 2 was higher than that in the other two trials before the onset of exogenous feeding. However after the larvae started active feeding and until the end of rearing period the increase in weight in trial 1 was higher than that in the other two trials. Therefore we can conclude that growth is independent of stocking density in the early stages since the yolk sac provides nutritional needs of larvae. The three trials showed no significant difference with each other ($p>0.05$). However after the onset of exogenous feeding a significant difference was observed in trials with lower stocking density. This was because this stage of growth depends on the amount of food the fish consumes from its environment (Helser *et al.*, 1997).

High stocking density can cause the decreasing of the feeding surface and also unequal distribution of food among larvae. Decrease in feeding surface results in crowding of larvae at a particular spot and this brings the decreasing of growth (Nazari, 1996), wounds, body injury and breaking of fins (Mokhaier, 1988). Active swimming also decreases the effectiveness of food convertion and biomass (Sattari *et al.*, 1970).

Morphological investigation of larvae after the onset of exogenous feeding also revealed that cannibalism was also significantly higher in trials with high stocking density.

Extensive studies have been conducted for different species (Siberian, Russian, sterlet, stellate and great sturgeon). Results from these studies showed that increase in stocking density of larvae in tanks resulted cannibalism when the larvae pass over to external feeding. Thus we can conclude that increase in stocking density resulted increased competition for food among larvae. This will lead to a variation in feeding levels and thus, results different weight classes among the larvae. Low stocking density provides sufficient feeding space for each fish and decreases the intensity of cannibalism. Studies conducted on the effects of stocking density on the increase in cannibalism in bony fishes such as carp also showed similar results.

Appearance of various deformities damaged fins, bent spine, hanging and looped tail, change in form of yolk sac etc., were lower at lower stocking density.

Thus it can be stated that the appearance of morphological deformities is not only the result of heavy metals and pesticides pollution, unfavorable feeding conditions, decrease in feeding level, imbalanced food, deficiency in starter diets, intra breeding, imbalance in vitamins, hereditary and genetic factors, mechanical injuries, loss of eggs during embryonic development and diseases, but also due to the unfavorable environmental conditions including stocking density.

Researchers believe that one of the most important reasons for the appearance of such deformities is the unsuitable rearing condition in the rearing tanks at the time of switching to exogenous feeding. When normal stocking densities are not used due to the presence of larval teeth, larvae are subjected to injury. However in the case of certain deformities such as bent spine, it is believed that impacts of genetic, feeding and environmental factors are more effective than stocking density (Leary *et al.*, 1991).

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