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SHORT COMMUNICATION

Effect of different fertilization and egg de-adhesion methods on the artificial propagation of Siberian sturgeon

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Abstract. In the present study, the effects coelomic fluid removal method (rinsing with clear water versus siphoning) and de-adhesion substances (milk solution, starch suspension and urea- NaCl-tannic acid solutions) had on fertilization rates and hatching success and time of Siberian sturgeon, *Acipenser baerii* Brandt were compared. It was concluded that 1) rinsing Siberian sturgeon eggs prior to fertilization is recommended when there is abundant, viscous coelomic fluid; 2) de-adhesion with milk or urea-NaCl-tannic acid is less time-consuming and results in higher, although not significantly, hatching percentages than those obtained with starch, and 3) de-adhesion with milk ensures the shortest and most synchronous hatching of Siberian sturgeon larvae. All of these issues require further experimental investigations.

Keywords: Siberian sturgeon, wet fertilization, semi-dry fertilization, egg de-adhesion

The siberian sturgeon, *Acipenser baerii* Brandt, is one of the most common species of sturgeons used in aquaculture worldwide (Hamlin et al. 2006). Although its artificial propagation and larval rearing techniques are well described and reliably documented (i.e., Gisbert and Williot 2002, Marturano et al. 2007), there have been no comparative studies on egg treatment effects prior to and after fertilization.

Since female sturgeon have a special oviduct structure, eggs cannot be efficiently hand-stripped. Eggs collected either from sacrificed females or removed with surgery techniques from live fish (Podushka 1999, Burtsev et al. 2002) are mixed with coelomic fluid, which can hinder the efficiency of fertilization and, consequently, it should be drained from the eggs before insemination (Dettlaff et al. 1993). However, the removal of this fluid by decantation, straining, or siphoning is hindered by its amount and viscosity.

Another problem encountered in hatchery procedures of sturgeons is egg adhesiveness, which must be eliminated prior to incubation (Dettlaff et al. 1993). To deactivate the adhesive layer of the fertilized sturgeon eggs silk, mud, fuller's earth, chemicals (urea-NaCl-tannic acid) and other treatments are widely used (i.e., Conte et al. 1988, Bouchard and Aloisi 2002). However, the effects of these techniques on the hatching success of Siberian sturgeon eggs are poorly documented.

Therefore, in the present study, the effects two methods for coelomic fluid removal and three de-adhesion procedures had on fertilization rate and hatching success and time of this species were compared.

In the middle of April, three female (body weight 13, 9.2, 9.7 kg) and four male (body weight 6.5, 7.5, 8.5, 9.5 kg) Siberian sturgeons were transported to the hatchery. The fish were kept at 17.1±0.6°C, and both sexes were injected intramuscularly with the gonadotrophin-releasing hormone analogue Gonazon (Intervet International BV,

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Boxmeer, The Netherlands) in a single dose of 40 μg kg^{-1} body weight. Before stripping, the fish were anesthetized in clove oil at a dose of 0.05 ml L^{-1}. Milt was stripped from the males between 27 and 31 hours after the hormonal injection and was stored at 8°C until fertilization. Eggs were obtained between 28 and 31 hours after injection from two females using the Podushka (1999) method. Prior to fertilization, the eggs from each female were divided into two groups, and the coelomic fluid was removed either by quick (about 15 s) rinsing in water, or by carefully siphoning it with a syringe. Each group of eggs was fertilized with the pooled milt of all males diluted in water at a proportion of 1:200 (Dettlaff et al. 1993).

These groups were further divided into three subgroups, and the following de-adhesion substances were used: milk solution (M), starch suspension (S), and chemicals (C). In the M treatment 7 L of water were added to 1 L milk with a 3.5% fat content. The S suspension was prepared by dispersing 5.0 kg potato starch in 10 L of water. In the C treatment, 40 g of NaCl and 160 g of urea were dissolved in 10 L of water and, finally, 0.25 ppt tannic acid solution was used for 3x20 seconds (Woynarovich and Woynarovich 1980). The eggs in all the treatments were stirred gently and slowly with a feather, and the solutions were changed at approximately 15-20 min intervals to maintain temperature and dissolved oxygen levels. The de-adhesion procedure was continued until the eggs did not stick to the fingers or to each other when lifted from the bowl. De-adhered eggs were incubated in 8 L Zuger jars at 19.1±0.9°C and were disinfected twice daily for 10 min with a 100 ppm formalin solution.

A random egg sample (200-300 eggs) was collected at neural tube closure to determine the proportions of normally fertilized eggs. When the first free-swimming larvae were noted, more random egg samples (150-250 eggs per de-adhesion treatment) were stocked into separate containers (20 L each). The hatched larvae were collected and counted at one hour intervals. Hatching success was determined by the proportion of hatched larvae from fertilized eggs. Unfertilized eggs removed to prevent fungal infections were not included in calculations of hatching rates.

The fertilization and hatching rates noted for the different treatments were compared with the Chi-square procedure and the data were analyzed separately for each female. When the effects of treatments were significant, pair-wise comparisons were performed.

Rinsing eggs before fertilization improved fertilization rates in both females, but especially in the second (Table 1). Visual examination indicated that the eggs of the female that ovulated earlier had less abundant and less viscous coelomic fluid than did those of the second female. Thus, the current results concur with those reported by Van Eenennaam et al. (2008) who reported that rinsing enhances the fertilization of green sturgeon, *Acipenser medirostris* Ayres, by effectively removing viscous coelomic fluid. These authors also suggested that exposing the eggs

Table 1

Fertilization and hatching success* of Siberian sturgeon eggs after rinsing or siphoning the coelomic fluid and de-adhering eggs with different substances

	Rinsed			Siphoned		
	Milk	Starch	Urea+NaCl, tannic acid	Milk	Starch	Urea+NaCl, tannic acid
Fertilization rate (%)						
Female 1	89.55^{a}	79.66^{ab}	79.54^{ab}	66.67^{b}	79.03^{ab}	76.47^{b}
Female 2	85.89^{a}	88.75^{a}	87.69^{a}	61.53^{b}	69.11^{b}	66.67^{b}
Hatching rate (%)**	95.40^{bc}	98.21^{c}	93.95^{bc}	98.46^{c}	85.85^{a}	90.90^{ab}

*Values with different superscripts in the same row are significantly different. Chi-square = 11.35 with 5 degrees of freedom ($P < 0.05$; $\chi^2_{crit.} = 11.1$)

**Hatching rate was determined from fertilized eggs only from female 1

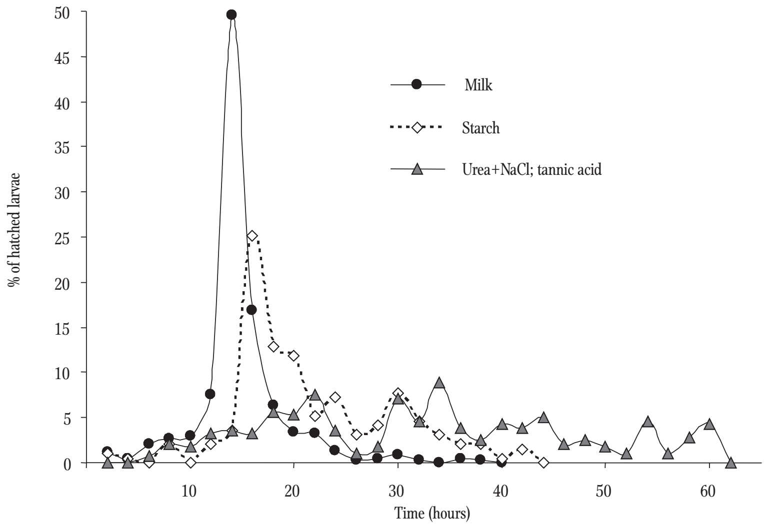

Figure 1. Hatching dynamics of Siberian sturgeon larvae over the hatching period after different egg de-adhesion methods were applied.

to freshwater before fertilization enhanced gamete interaction by stimulating the eggs to release a sperm attractant substance.

The elimination of egg adhesiveness required about 40 min for the M and C and 60 min for the S treatments. The hatching rate was generally higher for rinsing than for siphoning the coelomic fluid, but the difference between the pooled data (per fluid removal method) was not significant (Table 1). Hatching occurred between 84-122; 86-126, and 89-144 hours after fertilization for the M, S, and C treatments, respectively. These periods were apparently not influenced by the fluid removal methods, but were strongly affected by de-adhesion treatments. The shortest and most synchronous hatching was achieved with milk treatments followed by starch and chemicals (Fig. 1). This feature can be explained by the susceptibility and permeability of egg membranes to different types of organic or inorganic materials dissolved or suspended in the water. Tannic acid concentration and exposure time should be investigated further since a study by Demska-Zakęś et al. (2005) indicated the hatching process of pikeperch, *Sander lucioperca* (L.), larvae after the inappropriate use of this substance.

Based on the results, it was concluded that 1) rinsing Siberian sturgeon eggs prior to fertilization is recommended when there is abundant and viscous coelomic fluid, 2) de-adhesion with milk, or urea-NaCl-tannic acid are less time-consuming and result in higher, although not significantly, hatching percentages than with starch and 3) de-adhesion with milk ensures the shortest and most synchronous hatching of Siberian sturgeon larvae. All of these issues further experimental investigations.

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Streszczenie

Wpływ zróżnicowanych metod zapłodnienia oraz rozklejania ikry na przebieg sztucznego tarła jesiotra syberyjskiego

Przedstawiono efekty usuwania kleistej otoczki poprzez spłukiwanie za pomocą czystej wody lub przemywania pod ciśnieniem oraz rozklejania ikry przy użyciu roztworu mleka, zawiesiny skrobi i mieszanki mocznika, soli oraz kwasu taninowego na procent zapłodnienia, wyklucia oraz czas inkubacji ikry jesiotra syberyjskiego *Acipenser baerii* Brandt. Stwierdzono, że w przypadku obfitego występowania kleistej otoczki wskazane jest spłukiwanie ikry jesiotra syberyjskiego przed zapłodnieniem. Rozklejanie ikry za pomocą mleka lub mieszanki mocznika, soli oraz kwasu taninowego jest mniej czasochłonne oraz skutkuje wyższym, chociaż nieistotnym statystycznie, procentem zapłodnienia w porównaniu do stosowania zawiesiny skrobi. Rozklejanie za pomocą mleka zapewnia najkrótsze oraz najbardziej zsynchronizowane klucie larw jesiotra syberyjskiego. Wszystkie poruszone kwestie wymagają dalszych badań.