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Analysis of the post-vitellogenic oocytes of three species of Danubian Acipenseridae

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ABSTRACT. Post-vitellogenic oocytes of beluga (*Huso huso* Linnaeus, 1758), Russian sturgeon (*Acipenser gueldenstaedtii* Brandt, 1883) and sterlet (*Acipenser ruthenus* Linnaeus, 1758), sampled downstream of the "Iron Gate II" dam on the Danube River, were characterised according to diameter, dry mass, water and protein contents. All oocytes examined were ovoid in shape with the major diameter being measured in the animal-vegetal axis. The beluga oocytes were the largest, with major and minor diameters of 4.35 ± 0.13 and 3.64 ±0.14 mm, respectively. The oocytes of the Russian sturgeon were the next largest, with major and minor diameters of 3.69 ± 0.16 and 3.36 ± 0.15 mm, respectively, while those of the sterlet were the smallest, with major and minor diameters of 2.40 ± 0.10 and 2.14 ± 0.07 mm, respectively. Values for oocyte wet and dry mass (mg/ind) ranged from 25.9-32.1 for wet mass and 12.2-15.5 for dry mass of the beluga oocytes, 18.9 ± 1.4, and 9.01 ± 0.12 for wet and dry mass of the Russian sturgeon oocytes, to 6.5 ± 0.3 and 3.07 ± 0.14 of the sterlet oocytes. The water content of the oocytes of all three sturgeons was very similar (51-53% of wet mass). The protein content (% of dry mass) was highly conserved among the species at 53.0 ± 2.0, 55.9 ± 3.8 and 50.0 ± 1.2 for the oocytes of beluga, Russian sturgeon and sterlet, respectively.

KEY WORDS : Russian sturgeon, beluga, sterlet, oocytes, Acipenseridae, yolk proteins

INTRODUCTION

The Acipenseriformes live almost exclusively in the Northern Hemisphere with half of the extant number of species occurring in Europe, mostly in the Ponto-Caspian region (Billard & Lecointre, 2001). Recently several associations have recommended that the status of beluga (*Huso huso*) be upgraded to Appendix I under current CITES listings since it has almost been extirpated from the Black Sea (Vecsei et al., 2002). Indeed many of the stocks of sturgeons have dramatically decreased, primarily as a result of over fishing and habitat deterioration. Habitat loss is predominantly caused by pollution and damming of rivers, which blocks migration and access to proper spawning grounds (STEVENSON & SECOR, 1999 ; Vecsei et al., 2002).

In Yugoslavia there was a significant drop in catch of all sturgeon species after 1970 and 1984, when the lower stretch of the Danube was dammed by, respectively, Djerdap I and II hydropower stations (JANKOVIC, 1993 ; VECSEI et al., 2002). Today, species of Acipenseridae, except sterlet, occur in Yugoslavia only in a 17.8 km stretch of the Danube River, from the Djerdap II dam to the border with Bulgaria, close to the Timok River mouth.

Presently sturgeon farming, outside of Yugoslavia, yields more than 2,000 ton per year and about 15 ton of caviar. Such efforts could contribute to a reduction of fishing pressure on wild stocks (BILLARD & LECOINTRE, 2001).

The most significant research on the biology and cultivation of sturgeons was conducted in the former Soviet Union (DETTLAFF et al., 1993). Since the collapse of the Soviet Union, however, virtually all research effort has ceased through lack of funding.

The situation is very much the same in the countries bordering the lower region of the Danube (Yugoslavia, Romania, Bulgaria and Ukraine). Despite the research effort of DETTLAFF et al. (1993), some problems regarding normal growth and success of fertilization of the oocytes and eggs still exist. According to AMIRKHANOV (1974) earlier studies suggested that protein concentration in oocytes can be a valid indicator of the staging, quality and successful fertilization and normal growth of the fertilized eggs (BRASHE, 1964 ; FEDOROVA & GRUDANOV, 1968). More recently, CHEBANOV (2001) conducted similar research on Russian sturgeon (*Acipenser gueldenstaedtii*) in which he followed protein and water contents in the oocytes over a ten year period. Together with other physiological parameters, these findings helped him to evaluate reproductive quality of the natural and artificially reared populations.

Consequently, the goal of this investigation was to make a preliminary comparative study of the biometry, gravimetry and protein contents of the oocytes of three species of Danube sturgeons : beluga, Russian sturgeon and sterlet (*Acipenser ruthenus*).

MATERIAL AND METHODS

All females were sampled during the spring and fall of 2001 in the Danube, downstream of "Iron Gate II" dam (863 km from the Danube delta). Oocytes were sampled from three beluga females (BeB, BeC, BeD) caught in April and May, total length (TL) = 282, 287, 304 cm and wet mass (W) = 161, 194.5, 159.6 kg respectively), one Russian sturgeon (GuA) caught in October, TL = 180 cm, W = 25 kg) and two sterlet (RuA, RuB) caught in April, total length (TL) = 64, 68 cm and wet mass (W) = 1.3, 1.5 kg. The second sterlet (RuB) had undergone final oocyte maturation and provided ovulated eggs.

Major (D_1) and minor (D_2) diameters were measured in 0.9% NaCl. Wet masses (WM) were determined after removal of excess ovarian fluid, and the samples were frozen and stored at –20°C until lyophilisation, dry mass and protein content analyses.

Oocyte volume (V) was calculated using the formula :

$$V = \frac{4}{3} \bullet \pi \bullet \frac{D_1^2}{2} \bullet \frac{D_2}{2}$$

where D_1 and D_2 represent the major and minor diameters respectively.

Oocyte wet (WM) and dry (DM) masses were measured to the nearest 0.1 mg. Lyophilization was performed for 48 hours.

Oocyte proteins were precipitated with 1 mL 6% TCA to remove free amino acids, then centrifuged (10,000 x *g*, 5 min, 4° C). The precipitate was washed once in 6% TCA, then solubilised in 1M NaOH. Solubilisation was accomplished with sonification (3 x 15 sec at 400 Hz). Prior to analyses, 1 mL double-distilled water was added to give a final concentration of 0.5M NaOH. Following the addition of the Lowry reagents (LOWRY et al., 1951), triplicate samples were read at 650 nm with a Pye Unicam spectrophotometer. Bovine serum albumin was used as standard.

RESULTS

The largest oocytes were obtained from beluga, and smallest from sterlet, while Russian sturgeon had intermediate sized oocytes (Fig. 1). The size classification was also evident for wet mass and dry mass, and reflected the size of the female caught. However, despite the differences in size, all oocytes had similar water content with values of 51-53% of wet mass. Similarly, the oocyte relative protein content (% of dry mass) was also conserved between the species and ranged from 50.0-55.9%.

Overall the data show that irrespective of size, the protein content and cell water are closely regulated components of the post-vitellogenic oocytes of the Danube sturgeons.

From one species only, the sterlet, we had the opportunity to sample oocytes (RuA) and ovulated eggs (RuB). From Fig. 1 it is possible to see that ovulated eggs of sterlet had bigger values for diameter, volume and protein content compared to the oocytes of the RuA female.

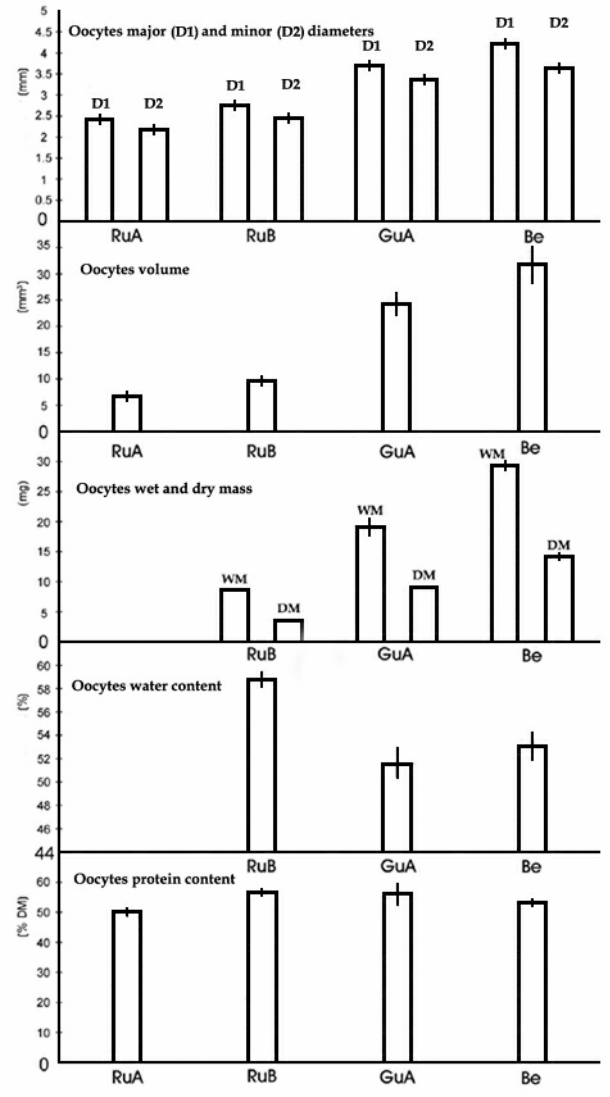

Fig. 1. – Figure 1. Major (D1) and minor (D2) diameter, volume, wet (WM) and dry (DM) mass, water and protein content of beluga (Be) oocytes, sterlet (RuA) oocytes, sterlet (RuB) eggs and Russian sturgeon (GuA) oocytes.

DISCUSSION

The data concerning oocyte biometry and gravimetry for three Danube sturgeon species were correlated with parental size. Beluga oocyte diameter, volume, wet and dry mass were the largest followed by Russian sturgeon, then sterlet.

Average diameters of three beluga oocytes were 4.20 mm for major and 3.64 mm for minor diameter. Data given by Holcik (1989) for this parameter were around 3.8 mm for major and 3.4 mm for minor diameter, which corresponded better to the data obtained for Russian sturgeon (3.7 and 3.4 mm). Between these two results are those (4.0 and 3.6 mm) obtained by Dettlaff & Ginsburg (1954). According to Berg (1949) 3.5 mm and 3.0 mm are average values for minor and major diameters respectively.

There are several data given for sterlet oocyte diameters by different authors. According to Holcik (1989) oocyte diameter is around 2.5 mm. Jankovic (1958) noted that oocyte diameters ranged from 2.0-2.9 mm for major axis and 1.8-2.8 mm for minor axis. Values obtained in the present study were 2.7 and 2.4 mm for the major and minor diameters of the ovulated eggs found in the abdomen of RuB, and 2.4 mm and 2.1 mm for the major and minor diameters of the post-vitellogenic oocytes of RuA.

Average wet mass of beluga oocytes was 29.3 mg, and 14.0 for its dry mass. Wet and dry mass for oocytes of Russian sturgeon were 18.9 mg and 9.0 mg, and 6.5 mg and 3.1 mg for sterlet oocytes. Chebanov (2001) found a difference in Russian sturgeon oocytes wet mass in the years 1991 - 2000. In that period, values ranged from 17.9 to 20.4 mg, which is very similar to results presented here.

Oocyte water and relative protein content of all three sturgeons were very similar. The water content results are at the lower range of 50-70 % reviewed by Kamler (1992) for freshwater fishes, and the protein content was similar to the lower range reviewed by Kamler (1992). Indeed the values found here for beluga are considerably lower than the recomputed 63% reported by Kamler (1992). Unlike the pelagic eggs of marine fishes (Finn et al., 2000 ; 2002a ; 2002b), sturgeon oocytes do not undergo proteolysis and hydration during final oocyte maturation (Finn et al., 2001 ; 2002c). Our findings suggest that, despite the difference in size of both female and oocyte, water content and protein levels are conservative aspects of the reproductive biology of sturgeons.

REFERENCES

AMIRKHANOV, G.A. (1974). The dynamics of free amino acid content during the embyonic development of the sturgeon, *Acipenser gueldenstaedtii*. *Vopr Ihtiol.* **19** : 127 –131.

BERG, L.S. (1949). *The freshwater fishes of the USSR and adjacent countries*. Akademia nauk USSR, Moscow & Leningrad.

BILLARD, R. & G. LECOINTRE (2001). Biology and conservation of the sturgeon and paddlefish. *Rev Fish Biol Fish* **10** : 355-392.

BRASHE, Z. (1964). *Biochemical embryology.* Inostr. Lit-ra, Moscow.

CHEBANOV, M.S. (2001). Ecological-technological principles of stock enchancement and living gene bank formation for conservation of Caspian and Azov Sea sturgeon diversity. *Report of Krasnodar Research Institute of Fisheries*.

DETTLAFF, T.A. & A.S. GINSBURG (1954). *The embrionic development of sturgeons (stellate sturgeon, Russian sturgeon, beluga) in connection with questions of their reproduction.* Acad. Sci. Press, Moscow.

DETTLAFF, T.A., A.S. GINSBURG & O.I. SCHMALHAUSEN (1993). *Sturgeon fishes development biology and aquaculture*. Heidelberg, Berlin, Springer-Verlag, New-York.

FEDOROVA, L.S. & S.D. GRUDANOV (1968). Some aspects of the protein metabolism in beluga females from the spring migration during gonad maturation. In : *Elaboration of the biological bases and biotechnics of rearing in sturgeon fisheries in bodies of water of the USSR*, Astrakhan.

FINN, R.N., H.J. FYHN, B. NORBERG, J. MUNHOLLAND, & M. REITH (2000). Oocyte hydration as a key feature in the adaptive evolution of teleost fishes to seawater. In : NORBERG, KJESBU, TARANGER, ANDERSON & STEFANSSON (eds), Proc 6th Int Symp Reprod Physiol Fish, Inst Mar Res & Univ Bergen, ISBN : 82-7461-048-2 : 289-291.

FINN, R. N., M. LENHARDT, A. FERRARA, P. CAKIC, J.J. ISELY, J. DEAN, M.S. EVJEN & H.J. FYHN (2001). Yolk proteins and free amino acids in Acipenseriformes. Abstract : Tenth European Ichthyological Congress in Prague 3-7th September 2001.

FINN, R.N., G.C., ØSTBY, B. NORBERG, & H.J FYHN. (2002a). *In vivo* oocyte hydration in Atlantic halibut (*Hippoglossus hippoglossus*). Proteolytic liberation of free amino acids, and ion transport are driving forces for osmotic water influx. *J. Exp. Biol*. 205 : 211-224.

FINN, R.N., M. WAMBOLDT, & H.J. FYHN (2002b). Differential processing of yolk proteins during oocyte hydration in fishes (Labridae) that spawn benthic and pelagic eggs. *Mar. Ecol. Prog. Ser.* 237 : 217-226.

FINN, R.N., M., LENHARDT, A., FERRARA, J.J., ISELY, J. DEAN, & H.J. FYHN (2002c). Yolk proteins and free amino acids in Acipenseriformes. Proc. 26th Larval Fish Conference of the American Fisheries Society. Bergen, Norway, 22-26 July.

Holcik, J. (1989). *Freshwater fishes of Europe. General introduction to fishes and acipenseriformes*. Aula Verlag, Wiesbaden.

JANKOVIC, D. (1958). *Ecology of Danubian sterlet* (*Acipenser ruthenus*). Institute for Biological Research, Belgrade.

JANKOVIC, D. (1993). Populations of Acipenseridae prior and after the construction of the HEPS Djerdap I and II. *Ichthyologia* **25** : 29-34.

KAMLER, E. (1992). *Early life history of fish. An energetic approach*. Chapman and Hall, Fish and Fisheries Series 4. London

LOWRY O.H., N.J. ROSEBROUGH, A.L. FARR & R.J. RANDALL (1951). Protein measurement with the folin phenol reagent. *J Biol Chem* 193 : 265-275.

STEVENSON, J.T. & D.H. SECOR (1999). Age determination and growth of Hudson River Atlantic sturgeon, *Acipenser oxyrinchus*. *Fish. Bull., U.S.* **98** : 153-166.

VECSEI, P., R. SUCUI & D. PETERSON (2002) Threatened fishes of the world : *Huso huso* (Linnaeus, 1758) (Acipenseridae). *Environ Biol Fish* 65 : 363-365.