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Stress indicators throughout the reproduction of farmed Siberian sturgeon *Acipenser baerii* (Brandt) females

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Abstract

We compared the profiles of plasma stress indicators (cortisol, lactate and glucose), ions (sodium, calcium, potassium, magnesium and chloride) and the reproductive potential of females' Siberian sturgeon (*Acipenser baerii*, B.) under two reproduction procedures. The treatment 1 had three steps: ovarian follicle sampling, further hormonal injection, and reproduction. Ovarian follicle sample and hormonal injection were performed simultaneously in treatment 2. To allow blood sample without handling, fish were cannulated. Basic levels of studied plasma components were established. A cortisol peak (~200 ng/ml) was recorded after the hormone injection without handling and was very similar to that observed post-handling. Surges of lactate (≥ 400 mg/l) were similar whatever the handling, ovarian follicle sampling with or without hormonal injection and reproduction. Glucose profiles were similar whatever the treatment. The return time of glucose content to resting level was longer than those for cortisol and lactate. Glucose profiles together with reproductive potential suggest that, in our experimental conditions, confinement did not have any detrimental consequences. Calcium content did not change. Potassium and magnesium decreased only slightly but significantly, so did sodium and chloride in a higher range. This leads us to suggest that the plasma lost in sodium and chloride might have been transferred to the coelomic fluid. The observed variations in all ion contents simultaneously with those of cortisol, suggest a correlation between cortisol and hydromineral balance. Reproductive potential of females were close to each other for both reproduction treatments (68.8% & 71.3%) but were significantly higher than those recorded in non-cannulated fish (42%).

Keywords: *Acipenser baerii*, Reproduction, Stress indicators, Cannulation, Ions

Introduction

In farming conditions, reproduction in sturgeon is induced by hormonal treatment (Charlon and Williot 1978). No external indices allow one to identify the fish ready to spawn. The best procedure is to remove some ovarian follicles for further observation (especially germinal vesicle migration and in vitro maturation competence test) (Kasanskij et al. 1978; Lutes et al. 1987; Williot et al. 1991; Dettlaff et al. 1993, Williot 2002).

As a result, handling of the fish is necessary, which may be considered as a stressful situation, possibly leading to worse reproduction results (Pickering 1993). Indeed in Siberian sturgeon (*Acipenser baerii*), some poor

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reproduction results have been recorded (Williot et al. 1991; Goncharov et al. 1999). In studies of the consequences of hypoxic stress on immature Siberian sturgeon, modifications in plasma concentrations of cortisol, lactate, sodium and potassium have been demonstrated (Nonnotte et al. 1993; Maxime et al. 1995). Thus, some stress-induced physiological modifications in Siberian sturgeon are similar to those reported in teleosts (Mazeaud et al. 1977). Cortisol has recently been confirmed to be the main glucocortocoids in *Scaphirhynchus albus* (Webb et al. 2007). High cortisol levels have also been positively correlated with reproduction success in chondrostean, *Acipenser stellatus* and *Huso huso* (Semenkova et al. 1999), *Acipenser transmontanus* (Lutes et al. 1987), and in teleosts such as *Oncorhynchus nerka* (Donaldson and Fagerlund 1968), *Pleuronectes platessa* (Wingfield and Grimm 1977), *Salmo trutta* (Pickering and Christie 1981), *Heteropneustes fossilis* (Lamba et al. 1983). Cortisol-fed *Ictalurus punctatus* exhibited 47% more spawns than control-fed fish (Small 2004).

Cortisol in teleosts is also suspected of acting on the regulation of Ca^{++} in goldfish (Van Der Kraak 1990, 1991) and is known to stimulate ion pumps as reflected by Na^{+} /K^{+} - ATPase activity of different tissues in freshwater trout (see review in Bern and Madsen 1992).

The present investigation compares the changes occurring in stress plasma components (cortisol, lactate, and glucose), ions (potassium, calcium, and sodium, magnesium and chloride) and reproductive potential in two groups of Siberian sturgeon breeders managed according to two reproduction procedures.

Materials and methods

Fish, preliminary handling and care

Fish were previously spawned 11-year-old tagged females. They were randomly chosen from pre-selected fish in November (Williot and Brun 1998). Fish were starved one day before first fish-handling in rearing raceways. Three days before the beginning of the study about 15 randomly chosen females were isolated at the head of the raceway to make them easily fishable. In order to establish the resting levels of the plasma characteristics, the fish were prepared as follows: at the beginning of the experiment, females were immediately blood-sampled in situ (in the raceways) in the caudal vasculature. This procedure lasts no more than 2 min and then is presumed to be representative of the resting level. The fish were carried in a well-oxygenated tank, anaesthetized and were blood-sampled again 8 min later upon arrival in the hatchery.

With the exception of the former blood sample to assess the resting level, before any further handling (ovarian follicle sample, cannula installation, and Eggs collection) fish were tranquilised for about 5 min in a bath containing clove oil (emulsified in ethanol; 1:10, clove oil/ethanol) added to water at a concentration of 40 ppm. Afterwards, fish were placed ventral side up on a V-shaped table with a continuous supply of water in the mouth allowing the fish breathing continuously (Doroshov et al. 1983).

Experimental design

Two reproduction procedures were applied: treatment 1 had three steps: sampling of ovarian follicles, hormonal injection, and reproduction; treatment 2 had two steps: sampling ovarian follicles and hormonal injection simultaneously and reproduction (Table 1A). Hormonal injections in treatment 1 did not require handling as they were practised directly (intra muscular) in fish moving in two m-diameter tanks. Fish were cannulated to enable successive blood sample without handling. Six experiments were carried out from late February till mid-April, each lasted a week and contained 2-4 cannulated hormone-injected fish according to the 2 aforementioned reproduction treatments, 0-1 cannulated and sham-injected fish, 4 fish without cannulation and hormone-injected, and 4 non-cannulated fish which were sham-injected. The last experiment contained only 3 non-cannulated fish (Table 1B). Cannulated fish were blood-sampled at the time of cannulation and then 1, 3, 6, 12 h later, at the observed ovulation time, at the end of reproduction and 6 to 7 h later. Non-cannulated fish were blood- sampled only at the normal handling time.

Maintenance of fish and induced reproduction

After cannulation and/or follicle sampling, each female was placed in an individual 2 m diameter tank (40 to 50 cm water depth) supplied with oxygen-saturated running water. The breeders were injected intra muscularly with an aqueous (0.9% NaCl) suspension containing 5 mg/kg of body weight of carp hypophysis powder purchased from Argent Chemical Laboratories (Redmond, WA, USA). Water temperature was maintained at 15 °C up to the end of

the experimental period. The hormone injections were performed at 23.00 ± 1 h to obtain the first oviposition two days later, early in the morning. Immediately after this work on the females, 8 to 10 males were injected with carp hypophysis powder at a rate of 2 mg/kg of body weight.

Ovulation can be detected in two ways. The most common is the presence of eggs in the bottom of the tank. The second is to observe the consequence of a gentle abdominal stripping, as we know by experience that some fish need to be helped to expel their eggs. This latter procedure was generalized and the corresponding handling took place a few hours after the shortest possible latency and was repeated if necessary (Williot et al. 1991). In every case, the collection of eggs (stripping and laparotomy) was carried out 4 h after the beginning of the ovulation, as it is known that this delayed collection gives the best fertilisation rates (Williot et al. 1991). Reproduction fish were placed in a V-shaped table with a constant supply of water in the mouth. Before working with the females, semen samples were collected and the best four, from a motility point of view, were kept in the refrigerator until fertilisation, at which time they were pooled and used for all batches of ovulated eggs.

Table 1. Protocol: A) Scheme of handling and blood sample schedule depending on spawning procedure in a given experiment

Spawning procedure	Operation and blood sample schedule
Treatment 1 (3 steps)	Ovarian follicle sample & cannulation (handling) — Hormonal injection (no handling) — Ovulation, Spawning (handling)
Blood sampling schedule (h)	0 1 3 6 12 — 32 33 35 38 44 50 56 62 68 72 79
Treatment 2 (2 steps)	Ovarian follicle sample, cannulation, hormonal injection (handling) — Ovulation, Spawning (handling)
Blood sampling schedule (h)	32 33 35 38 44 50 56 62 68 72 79

B) Total number of fish depending on treatment

Cannulation	Spawning procedure	Number of fish
Cannulated	Treatment 1 (3 steps)	8
	Treatment 2 (2 steps)	8
	Control (sham injected)	4
Non-cannulated	Treatment (2 steps)	23
	Control (sham injected)	23

Fish cannulation

First we carefully placed the cannula in the dorsal artery behind the genital papilla. For this we used a Tuhoy syringe (N° 15) in which one of the ends of a 2.5 m small polyethylene capillary (Cannula 3404, internal - external diameters are 0.76 and 1.22 mm, from Biotrol, France) was installed. When blood was seen entering the capillary, we gently sank the capillary 5 to 6 cm into the vasculature and then the syringe was taken out. The capillary was previously emptied of special Ringer solution (see next paragraph) and closed with a flame. The cannula was then carefully fixed in place with three small subcutaneous bridges and three cross stitches. The outside end of the cannula was attached to a float, allowing one to sample the fish without any disturbance. This operation together with the follicle sampling lasted half an hour.

Blood samples, preparation and analysis

For each sample, the first 0.5 ml blood fraction collected was discarded. After collecting 2 to 2.5 ml, the rest of the blood was carefully pushed back with an adapted Ringer solution (Salin 1992) composed of NaCl (130 mmol/l), KCl (2.71 mmol/l), $CaCl_2$ (1.94 mmol/l), $MgCl_2$ (0.9 mmol/l), $NaHCO_3$ (5 mmol/l), glucose (1 mmol/l) and Li-heparin (200 UI/ml) and then the cannula was again closed with a flame. The blood was collected in microtubes previously supplied with Li-heparin at a ratio of 20 µl of a solution containing 100 UI/ml for 1.25 ml of blood, and then immediately centrifuged at 8000 rpm for 8 min. Plasma was then separated into two different batches. 500 µl for cortisol and ion analysis and 200 µl for lactate and glucose analysis after adding NaF at a ratio of 2mg/ml to stop the activity of glycolitic enzymes. The batches were stored at +4 °C for analyses in the following days. Cortisol was then determined by Enzyme Linked Fluorescent Assay (VIDAS from BioMérieux, France) (to the nearest ng/ml). Lactate and glucose were determined by enzymatic methods, PAP from BioMérieux and GOD/PAP test (Randox Laboratories Ltd, Crumlin, UK), respectively. Na^+ and K^+ concentrations were determined by light photometry on Ionocal 120 (referred to lithium from Hycel). Cl^- was measured by colorimetry after mercuric-thiocyanate reaction (BioMérieux, France). Ca^{2+} and Mg^{2+} were determined by colorimetry after blue-methylthymol indicator reactions and calmagite in presence of EGTA respectively (BioMérieux, France).

Ovarian follicle observations

Ovarian follicle sampling was performed via an opening obliquely in the abdominal wall between the 3rd and 4th ventral scute (from the vent) slightly lateral to the mid-line. The tool used was a sharp cylindrical probe. Then a hollow probe introduced in the opening allows to sample a piece of developed gonad (Williot et al. 2005). Ovarian follicle sampling lasted about 10 min. We determined the mean diameter of the follicles, the advancement of the migration of germinal vesicle or polarisation index (Kasanskij et al. 1978) and the 50% time course (ET_{50}) of *in vitro* maturation competence recorded by germinal vesicle break down (GVBD) of follicles.

They were incubated at 18 °C in normal atmosphere in small capped petri dishes (55 mm in diameter) containing 7.5 ml of the SIS medium: NaCl (127.5 mmol/l), KCl (2.7 mmol/l), $CaCl_2$, $2H_2O$ (1.95 mmol/l), $MgCl_2$, $6H_2O$ (0.85 mmol/l), Na_2SO_4 (0.7 mmol/l), hepes (20 mmol/l) buffered at pH 7.7 (Williot et al. 1991) in the presence of progesterone (17,20β-dihydroxyprogesterone at 1 µg/ml of medium).

Reproductive potential

Reproductive potential was determined by counting the normal embryos at the end of gastrulation, small yolk plug stage (Dettlaff et al. 1993) for approximately 300 eggs, without anti-adhesive treatment, incubated in a large petri dish (90 mm in diameter) in our normal 12 °C closed UV treated water system. The small yolk plug stage proved to be a good assessment of further reproductive success (rates of hatching, normal larvae, and first feeding larvae) in case of normal incubating and larval rearing conditions (Dettlaff et al. 1993; Williot 1998).

Statistical analysis

Comparisons among treatments were performed by Kruskall Wallis ANOVA on ranks followed by the complementary Dunn's test (multiple comparison *vs* control in case of unequal sample size, cf results section) according to Scherrer (1984). Normality (Kolmogorov-Smirnov) and equal variance tests were computed.

Recorded data were computed with Sigma STAT software. The effective time to get 50% of maturation (ET_{50}) was calculated (SYSTAT software) by maximising the following classical log likelihood function according to Finney (1971):

$$L = \sum_{i=1}^{k} (GVBD_i \log(p_i) + GV_i \log(1 - p_i))$$

$GVBD_i$ = number of *GVBD* for time i
GV_i = number of GV for time i
p_i = zcf (a×(log(time$_i$) - log(ET_{50})))
where: p_i is the probability of GVBD for one follicle
zcf is the normal cumulative distribution
a is a constant.

In some cases, it is necessary to use penalties to force the program to move away from estimates which produce overflows. Values are presented as Mean ± SEM and significant level is $P < 0.05$.

Results

Due to loss of cannula during the reproduction procedure, the data of 2 treatment 1-females were not included. Data within treatments and controls (cannulated sham-injected fish) were pooled and plotted in Figures 1-7. Results from the non-cannulated fish are not shown, except for the basic levels, which are included in figures.

Cortisol

The profiles of the two-treatment females are very similar, exhibited two peaks, and highly significantly ($P < 0.01$) different from the control. The first and highest peak (~200 ng/ml) occurred 3 h after the hormonal injection without fish handling (treatment 1) or cannulation and hormonal injection (treatment 2) (Fig. 1). The second peak, at around 130 ng/ml, followed the reproduction procedure.

Cortisol content of treated females recover the resting level closed to basic level (7 ng/ml) 18 to 22 h after the hormonal injection and 10 h after reproduction. The profile of the control fish decreased rapidly post cannulation as for treatment 1-females and remained close to the basic level even after the sham injection of saline solution. The figures for non-cannulated females exhibited a post-reproduction peak at a level of 110 ng/ml. The profiles for non-cannulated sham-injected females remained at a very low level, not significantly different from the basic level.

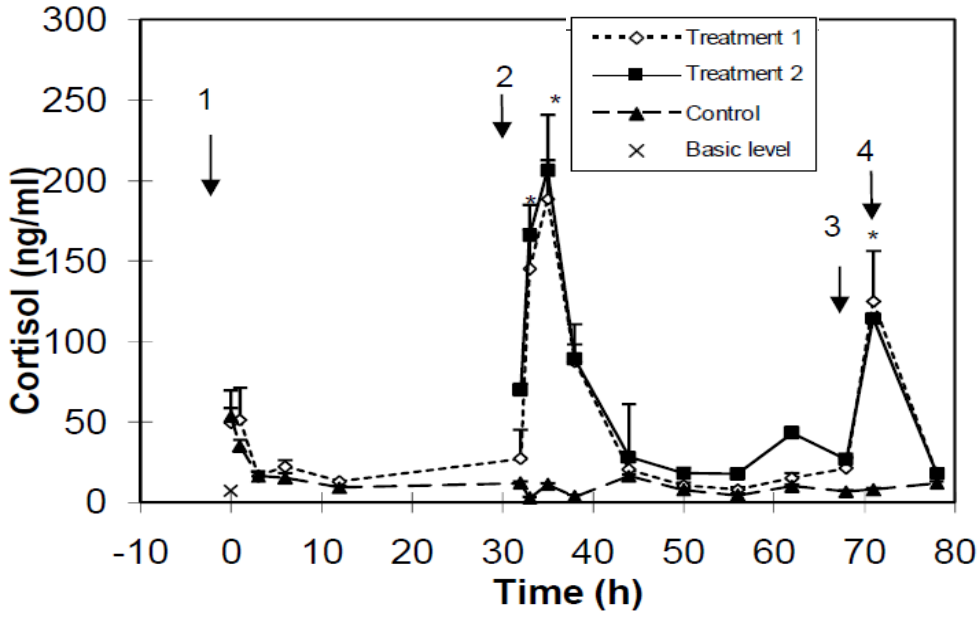

Fig. 1. Profiles of cortisol throughout the spawning of Siberian sturgeon (mean ± SEM). Cannulated females; arrows 1= cannulation and follicle sample for treatment-1 spawning females; 2= either cannulation, follicle sample and hormonal injection for treatment-2 spawning females or hormonal injection without handling for resting females or saline injection without handling for control female; 3= ovulation and 4= post-spawning. Points with the same symbol are not significantly different. Points with the asterisk are significantly different from control.

Lactate

Lactate profiles showed a sharp peak ≥ 400 mg/l after either handling. Therefore, there is no surge post hormonal injection in treatment 1-females (Fig. 2). The peaks appeared 3 h post-manipulation and a return 6 h post-manipulation closed to the basic level of nearly 30 mg/l was observed. The lactate content of control sham-injected fish decreased following the post-cannulation peak and then was closely at a very low level throughout the experiment. An increase to 160 mg/l occurred after the first manipulations of non-cannulated females. Another surge (~290 mg/l) followed the reproduction and further decreased to reach values no different from the basic level.

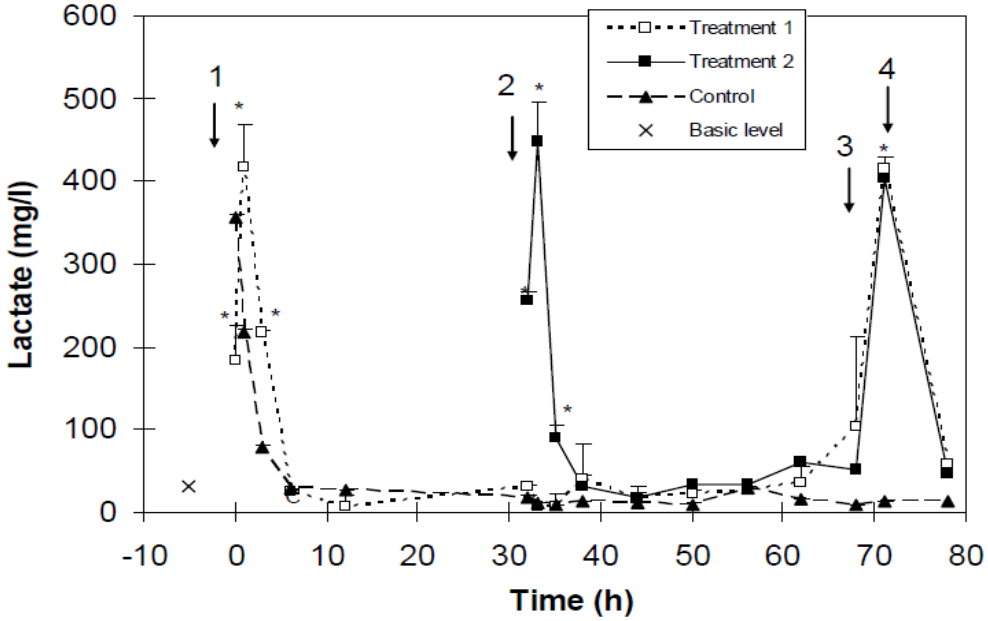

Fig. 2. Lactate profiles throughout the spawning of Siberian sturgeon (mean ± SEM). Cannulated females; arrows 1= cannulation and follicle sample for treatment-1 spawning females; 2= either cannulation, follicle sample and hormonal injection for treatment-2 spawning females or hormonal injection without handling for resting females or saline injection without handling for control female; 3= ovulation and 4= post-spawning. Points with the same symbol are not significantly different. Points with the asterisk are significantly different from control.

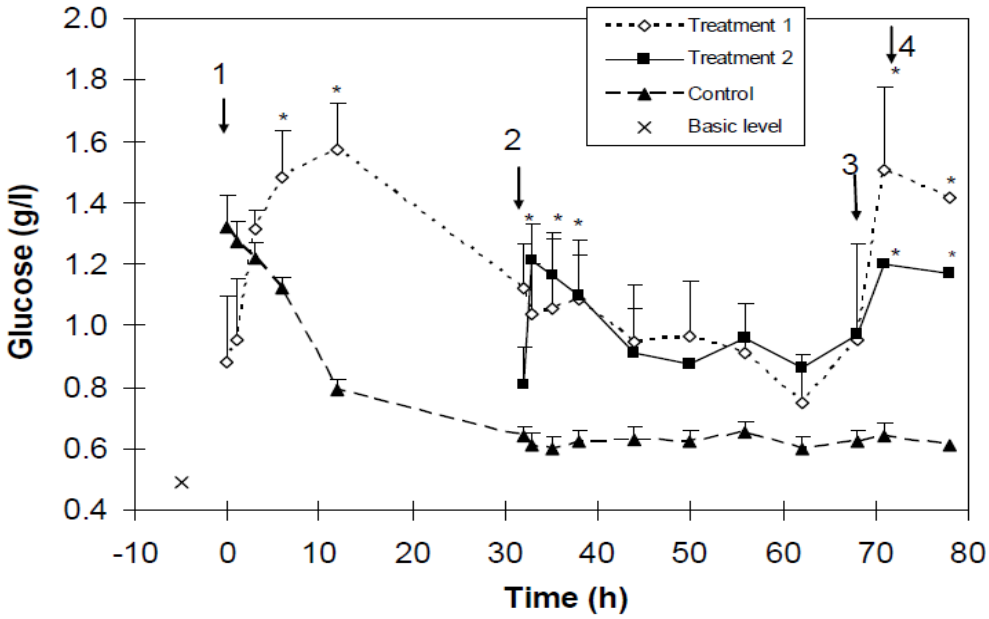

Fig. 3. Glucose profiles throughout the spawning of Siberian sturgeon (mean ± SEM). Cannulated females; arrows 1= cannulation and follicle sample for treatment-1 spawning females; 2= either cannulation, follicle sample and hormonal injection for treatment-2 spawning females or hormonal injection without handling for resting females or saline injection without handling for control female; 3= ovulation and 4= post-spawning. Points with the same symbol are not significantly different. Points with the asterisk are significantly different from control.

Glucose

The profiles of glucose of both treatment-females were similar and highly significantly ($P < 0.01$) different from the control (Fig. 3). There were two peaks post cannulation and reproduction. There maximum were similar within each treatment females, (~1.5 g/l) in treatment 1-females 3-6 h post cannulation and 1-2 h for treatment 2-females. The recovery level closed to basic level (~0.75 g/l) was reached 60 h post cannulation in treatment 1-females.

The control sham-injected fish profile showed a decrease following cannulation and recovery to a level close to the basic level some 30 h post-cannulation. The glucose profiles for non-cannulated injected females showed an increase up to reproduction even later, the end value (~1.3 g/l) being of the same magnitude as those recorded in cannulated fish. The glucose evolution for non-cannulated control females remained at a level, not significantly different from the basic level.

Ionic profiles

The initial levels established were as follows: Na^+ (132 ± 0.3 meq/l); Ca^{2+} (4.34 ± 0.08 meq/l) ; K^+ (2.60 ± 0.04 meq/l); Mg^{2+} (1.62 ± 0.03 meq/l) ; Cl^- (110.4 ± 0.3 meq/l).

The sodium profiles of two-treatment females showed some changes with a general significant decreasing trend ($r = 0.83$; $P < 0.01$) which was more pronounced for treatment-2 females ($r = 0.90$; $P < 0.01$) (Fig. 4). As a result, final values were lower than the initial ones, 16.5 meq/l and 19 meq/l for treatment-1 and treatement-2 females, respectively. The levels for control fish remained constant throughout the experiment. The profile for non-cannulated control females (not shown) exhibited a significant decreasing trend. The non-cannulated sham-injected fish profile showed that sodium did not vary. For the non-cannulated control females, a general decreasing linear trend ($r = 0.90$; $P < 0.01$) was shown and corresponded to a loss of 15 meq/l. The profiles for non-cannulated sham-injected females did not changed significantly.

Calcium did not show any significant variation (mean level of 4.5-4.7 meq/l) throughout the experiments whatever the treatment and the cannulation (presence or absence) (not shown). The calcium profile of control fish was fairly constant close to the basic level (4.3 meq/l).

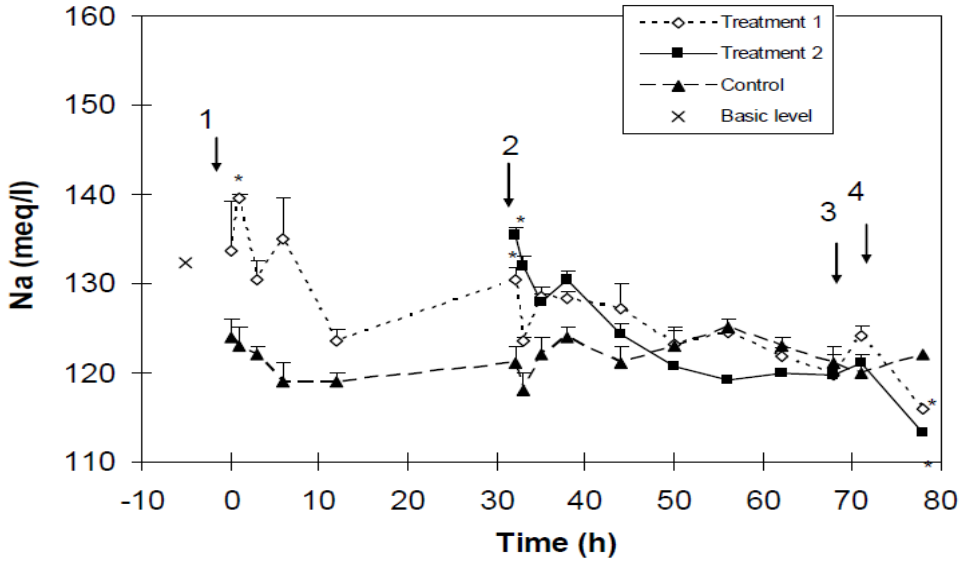

Fig. 4. Sodium profiles throughout the spawning of Siberian sturgeon (mean ± SEM). Cannulated females; arrows 1= cannulation and follicle sample for treatment-1 spawning females; 2= either cannulation, follicle sample and hormonal injection for treatment-2 spawning females or hormonal injection without handling for resting females or saline injection without handling for control female; 3= ovulation and 4= post-spawning. Points with the same symbol are not significantly different. Points with the asterisk are significantly different from control.

The potassium profiles showed some changes with a significant surge (~2.9 meq/l) at reproduction for both treated-females (Fig. 5). The levels for control fish fluctuated in the of 2.1-2.4 meq/l. The profile for non-cannulated females (not shown) was very similar to that described for the treated cannulated group with a surge at reproduction. The control fish profile showed that potassium did not vary.

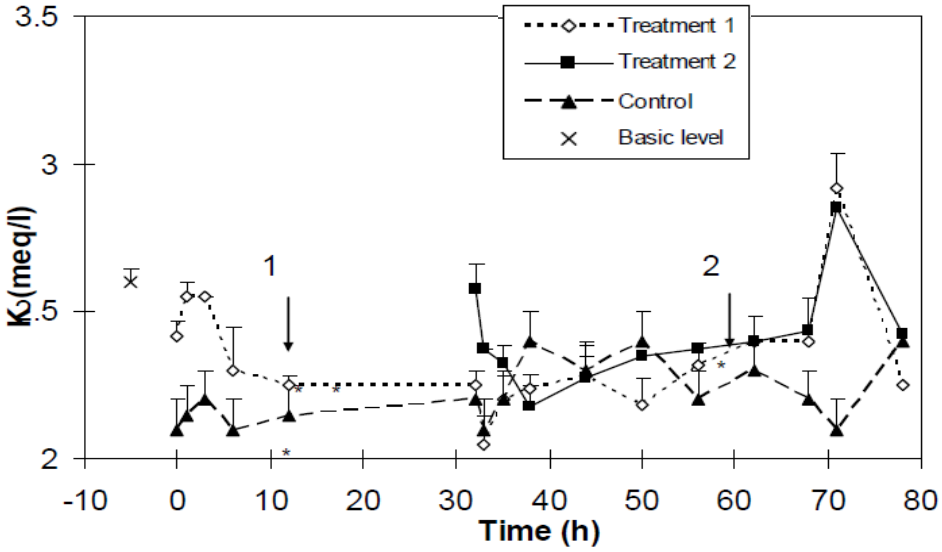

Fig. 5. Potassium profiles throughout the spawning of Siberian sturgeon (mean ± SEM). Cannulated females; arrows 1= cannulation and follicle sample for treatment-1 spawning females; 2= either cannulation, follicle sample and hormonal injection for treatment-2 spawning females or hormonal injection without handling for resting females or saline injection without handling for control female. Points with the same symbol are not significantly different. Points with the asterisk are significantly different from control.

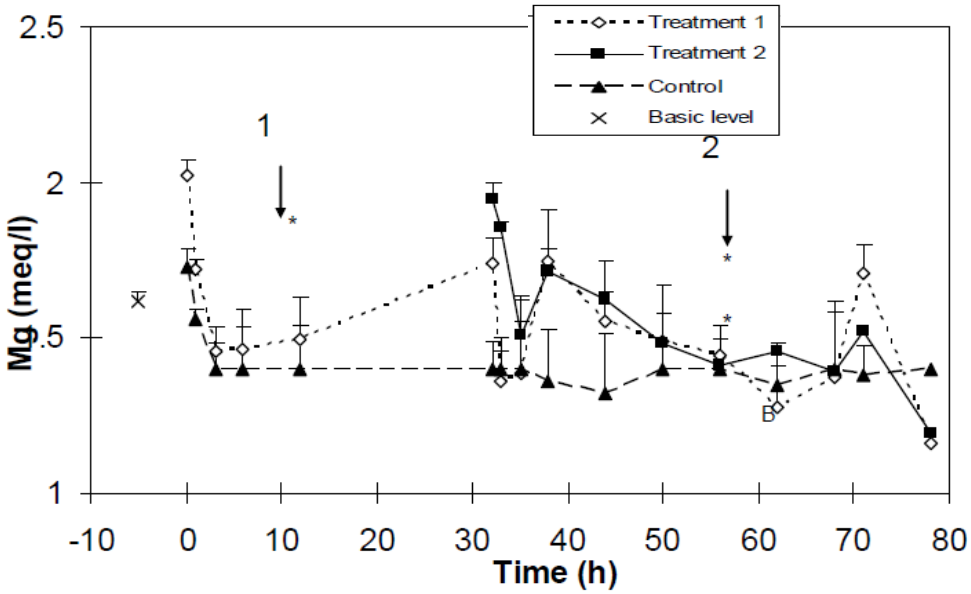

Fig. 6. Magnesium profiles throughout the spawning of Siberian sturgeon (mean ± SEM). Cannulated females; arrows 1= cannulation and follicle sample for treatment-1 spawning females; 2= either cannulation, follicle sample and hormonal injection for treatment-2 spawning females or hormonal injection without handling for resting females or saline injection without handling for control female. Points with the same symbol are not significantly different. Points with the asterisk are significantly different from control.

The curves for magnesium were similar for both treated females (Fig. 6). After cannulation and hormonal injection, we observed a decrease in magnesium level followed by an increase, and an increase after ovulation. After cannulation and hormonal injection, we observed a decrease in magnesium level followed by an increase, and an increase after ovulation followed by a decrease. The final values were slightly lower than the basic level. A general decreasing linear trend was shown by treatment-1 females ($r = 0.82$; $P < 0.01$). The profile for the control fish, after a rapid decrease post-cannulation, remained at the lowest level for all experimental fish. The profile for non-

cannulated control females was similar to that for the cannulated treatment-1 females and showed a slight decrease; that of the control fish remained at a constant level.

The chloride profiles of both treatment females were very similar with two peaks post cannulation and ovarian follicle sample (Fig. 7). In both cases, a general decreasing linear trend was observed, ($r = 0.90$; $P < 0.01$) and ($r = 0.82$; $P < 0.01$) for treatment-1 and treatment-2 females, respectively, leading to a respective loss in chloride of 15.5 and 20 meq /l as compared to the basic level. After a decrease post-cannulation, the chloride level of control fish remained fairly constant at about 100 meq/l. Concerning the non cannulated controlled females (not shown), the profile showed a general linear decrease ($r = 0.96$; $P < 0.01$) which corresponded to a loss of chloride of 12 meq/l in contrast to the non-cannulated control females, for which the chloride level remained unchanged.

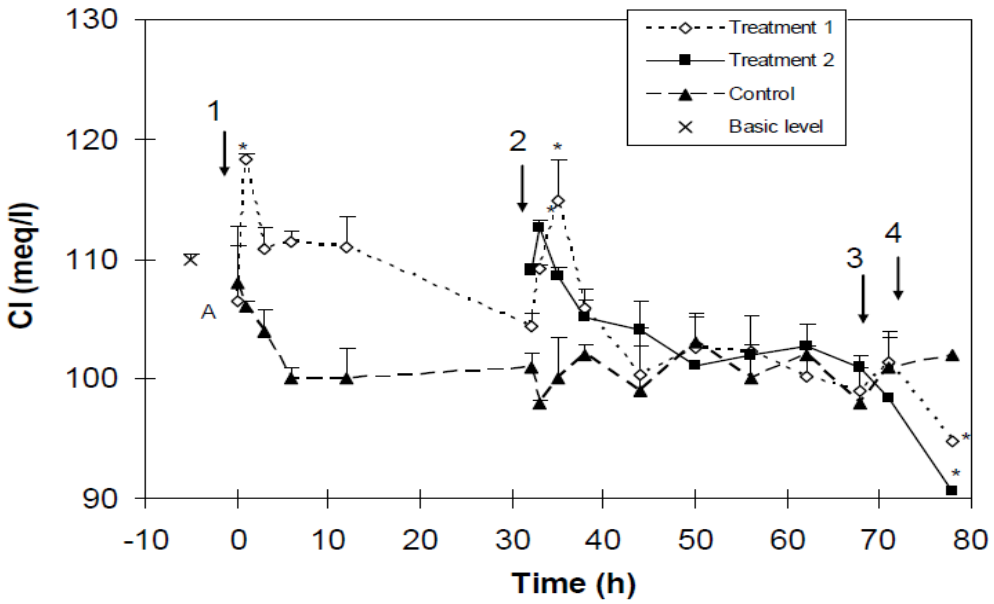

Fig. 7. Chloride profiles throughout the spawning of Siberian sturgeon (mean ± SEM). Cannulated females; arrows 1= cannulation and follicle sample for treatment-1 spawning females; 2= either cannulation, follicle sample and hormonal injection for treatment-2 spawning females or hormonal injection without handling for resting females or saline injection without handling for control female; 3= ovulation and 4= post-spawning. Points with the same symbol are not significantly different. Points with the asterisk are significantly different from control.

Reproductive potential

Weight, follicle diameter and polarisation index were similar whatever the experimental groups of females (Table 2). The value of ET_{50} was significantly higher for the treatment-1 females than for the treatment-2 and non-cannulated controlled females. None of the sham-injected (cannulated or not) females ovulated. The reproductive potential of treated females, all similar at around 70%, were significantly higher ($P < 0.05$) than that (42%) recorded for non-cannulated females (Table 2).

Table 2. Morpho-physiological characteristics and reproductive potential in Siberian sturgeon females*

Group of fish	Weight (kg)	Follicle diameter (mm)	Polarisation Index (%)	ET_{50} (h)	Embryonic survival (%)
Cannulated Treatment-1 (n=6)	9 ± 1.3^a	3.4 ± 0.2^a	6 ± 0.4^a	16.2 ± 5^a	68.8^a
Treatment-2 (n=8)	8 ± 1.2^a	3.3 ± 0.1^a	3 ± 1.2^a	11.2 ± 2.2^b	71.3^a
Non-cannulated (n=23)	7.4 ± 0.5^a	3.5 ± 0.1^a	4 ± 0.8^a	12.1 ± 0.7^b	42^b

* Values are Mean ± SEM. In the same row, values with different superscript letter are significantly different.

Discussion

The main aims of the present study were to compare the plasma profiles of stress indicators, ions, and the reproductive potential throughout the reproduction of Siberian sturgeon according to two management procedures. Except in two fish, the cannulation allowed us to blood-sample the breeders, throughout the experiment, i.e. three days for the treatment-1 without handling the fish. Non-cannulated fish were included in the protocol.

The cannulation might be very useful for other investigations on sturgeon. Cannulation has already been used (Mazik et al. 1994; Waring et al. 1996; Di Marco et al. 1999; Belanger et al. 2001) but, to our knowledge, never throughout the reproduction of sturgeon.

Peaks of stress indicators occurred after handling in the following order, lactate, cortisol, and later glucose. Lactate peaks reached the same level (~400 mg/l) whatever the handling. All three indicators recovered their basic level shortly (lactate and cortisol) and before initiation of ovulation for glucose. There was no surge of lactate when fish were injected in the water (treatment-1 females). It has been shown that the responses of treated females were very similar whatever the studied criterion, stress indicators, ionic contents, and reproductive potentials.

Cortisol peaks reached 3 levels, 50, 200, and 130 ng/ml after cannulation (treatment-1 females), hormonal injection (treatment–1 females) and together cannulation, ovarian sampling and hormonal injection (treatment-2 females), and ovulation respectively. The first peak observed in treatment-1 females was of the same magnitude as that reported after acute deep hypoxia (30 ng/ml) (Maxime et al. 1995), transportation or decrease in water level in the holding tanks (35 and 40 ng/ml) in males *A.transmontanus* (Belanger et al. 2001), handling of juveniles *A.naccarii* (73-79 ng/ml) (DiMarco et al. 1999), and injection of 5 µM ACTH in males *A.transmontanus* (76 ng/ml) (Belanger et al. 2001). In the treatment-1 females, the second peak appeared 4 h after hormonal injection and reached the same level of some 200 ng ml^{-1}, similar to the first peak for treatment-2 females. This suggests an absence of cumulative effect in the cortisol content at this stage. Hypophysis powder is essentially made of proteins. Most of them (~80%) are biologically inactive (Burzawa-Gerard 1971). The main action of these proteins are gonadotropic, adrenocorticotropic, and thyreotropic. The last targets the thyroid gland; the second (so called ACTH) controls the synthesis of cortisol. The absence of cortisol surge in sham-injected fish, and the high level of cortisol recorded in the present study post hormonal injection (~200 ng/ml) which was 2.5 times higher than that got by direct injection of ACTH (Belanger et al. 2001). It suggests that the present peak of cortisol might be not solely the response of ACTH action of the carp hypophysis. Some correlation between corticosteroids and reproduction in sturgeon has been reported. The endocrine activity of the interrenal in *Acipenser gueldenstaedtii* intensified with anadromous migration (Pen'kova 1974), the plasma content of cortisol in *Acipenser stellatus* increased when entering a river from the sea (Barannikova et al. 1982) and the plasma cortisol prior to hormonal injection turned out to be higher in responsive White sturgeon (*A.transmontanus*) than in non-responsive fish (Lutes et al. 1987), and in stellate (*A.stellatus*) and beluga (*Huso huso*) sturgeons (Semenkova et al. 1999). Basic levels of cortisol recorded in the present study (~7 ng/ml) are very similar to those reported in *A.transmontanus* and *A.naccarii* with 8 and 9.5 ng/ml respectively (Belanger et al. 2001; Di Marco et al. 1999).

Glucose concentration demonstrated a rapid rise following the first operation (cannulation) as the consequence of the mobilisation of energy reserves in activating liver glycogenolysis and inhibiting glycolysis, as already mentioned for Siberian sturgeon (Maxime et al. 1995). Later on, glucose content declined slowly to its basic level a few hours before ovulation and then increased until reproduction. This could mean that the consequences of stress due to the operations disappeared a few hours before ovulation, which perhaps indicates that the confinement of these fish, of around 1.1 m total length in 2 m diameter tanks, has no detrimental effects on them.

Simultaneously to stress indicators, changes in sodium, potassium, magnesium and chloride were observed, so that a correlation between cortisol variation and hydromineral balance might be suggested as already mentioned by Bern and Madsen (1992).

The present basic ionic values agree well with those already published on adult sturgeon in *Acipenser gueldenstaedtii* (Natochin et al. 1975), *Scaphirhynchus platorynchus* (Hunn and Christenson 1977) and *Acipenser transmontanus* (McEnroe and Cech Jr 1987). Moreover, the range of variation was very narrow, as a result, they may be considered as basic values. The loss of sodium is fairly well balanced by the loss of chloride, which could mean that reproduction (resumption of meiose, ovulation and reproduction) leads to a loss of circulating sodium chloride. Sturgeon ovarian follicles do not swell with maturation (Lutes et al. 1987) as observed in most of the teleosteans, moreover they became impermeable (Dettlaff et al. 1993). As a result, the changes observed in plasma ion characteristics in sturgeon during the final stages of reproduction may not be correlated with this follicle swelling phenomenon. It is known that sturgeon produce cœlomic fluid (Dettlaff et al. 1993), probably to facilitate

the expulsion of ovules through the oviducts. The osmotic pressure of Siberian sturgeon's cœlomic fluid ranges from 8 to 18 mosmol/l (Williot, unpublished data) and might explain, at least in part, the loss of plasma sodium chloride reported in the present work.

Morphological characteristics, weight, follicle diameter and polarisation index were the same for all groups of fish, treatment-1 and treatment-2 cannulated fish and non-cannulated reproduction fish. Both groups of cannulated fish differed from the *in vitro* maturation time-course; the values of the treatment-1 managed group were close to the upper limit of this time-course, which should lead to good fertilisation rates (Goncharov and Williot 1999). In both these groups of cannulated fish, the reasonably good recorded embryonic survivals (around 70%) were significantly higher than those recorded for non-cannulated females (42%). The absence of difference in reproductive potential between the two management schedules with cannulated females makes us more confident in using either procedure in future investigations.

Present findings suggest the same conclusion as aforementioned correlations between cortisol and reproduction in sturgeon (Barannikova et al. 1982; Lutes et al. 1987; Semenkova et al. 1999). Despite all the suggestions of correlations or putative correlations, no cause-effect relationship between cortisol and reproduction was shown. Some recent works focused on stress-related investigations were performed on young juvenile green sturgeon (*Acipenser medirostris*). They dealt with either chronic sturgeon stress (Lankford et al. 2003, 2005) or on the manipulation of interrenal response during stress (Lankford et al. 2006). The latter provided a useful tool in showing that fish immunized against green sturgeon ACTH attenuated significantly the cortisol secretion.

Additionally, the present findings and the related stress sturgeon literature suggest that stress could be regarded as differently depending on physiological stage of the fish; juveniles and breeders. When the former showed classical changes in physiological characteristics of the stress (whatever the cause of stress), there are suggestions that reproduction might be a stressful state in the very last steps of reproduction. However, the potential physiological role of classical stress indicators at reproduction is still to be investigating. Present findings agree well with those of Bayunova et al. (2002) who reported that short term hatchery practises do not lead to deterioration of gamete quality in sturgeon in contrast with long storage at high densities at a water temperature close to the spawning one. Moreover, Milla et al. (2009) in a recent synthesis on teleosts fish suggest a positive effect of cortisol, directly or indirectly, on oocyte maturation and ovulation. Additionally, present findings can be considered as a good example of allostasis suggested by Schreck (2010) to illustrate the positive effect of low stress level on reproduction.

This means that cannulation and either treatment could be applied without any detrimental effects on the reproductive capacities of the females. A similar result was reported for *Siganus guttatus* (Ayson 1989), and to explain this, the author suggested that corticosteroids might be involved.

In conclusion, the present design allowed us to show for the first time in reproductive female sturgeon simultaneous profiles for cortisol, glucose, lactate and main ions and to establish the basic levels of these components. Cortisol peaks are correlated with temporary fluctuations in ions post-hormone injection. The fairly important loss of sodium chloride might be related to the cœlomic fluid. No differences appeared between the two groups of cannulated females and the current confinement seemed not to have any detrimental effects. The fact that the better reproductive potential was recorded in cannulated fish remained speculative except considering stress as a normal component of reproduction and/or that low level of stress may positively impact the reproduction.

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