ELSEVIER

Comparative Biochemistry and Physiology Part A 121 (1998) 351–354

CBP

Serum parameters of Adriatic sturgeon *Acipenser naccarii* (Pisces: Acipenseriformes): effects of temperature and stress

E. Cataldi [a,*], P. Di Marco [a], A. Mandich [b], S. Cataudella [a]

[a] *Dipartimento di Biologia, Università 'Tor Vergata', Via della Ricerca Scientifica, 00133 Rome, Italy*
[b] *Istituto di Anatomia Comparata, Università di Genova, Via Benedetto XV 5, 16132 Genova, Italy*

Received 24 March 1998; received in revised form 17 September 1998; accepted 18 September 1998

Abstract

Data on the concentrations of some blood constituents of captive Adriatic sturgeon, *Acipenser naccarii*, a primitive bony fish, are reported. Serum osmolality, Na^+, K^+, Cl^-, Ca^{2+}, cortisol, glucose and total protein concentrations were measured. The effects of anaesthesia, temperature, crowding and prolonged handling stress were tested on a group of 12 4-year-old sturgeons sampled repeatedly. The anaesthetic dose of MS 222 (140 mg l^{-1}) induced significant osmolality elevation in the sturgeon. After exposure to colder temperature (17 versus 25°C), cortisol and Cl^- concentrations significantly decreased. The cultured sturgeon did not seem susceptible to crowding and prolonged handling stress, since neither the serum cortisol and glucose levels nor the other blood parameters were affected by these stressors. Results are compared with the few available data on other chondrostean fish and with those on teleosts.

Keywords: Acipenser naccarii; Blood chemistry; Cortisol; Osmolality; Serum constituent; Stress; Sturgeon; Temperature

1. Introduction

Blood parameters are increasingly used as indicators of the physiological or sublethal stress response in fish to endogenous or exogenous changes. The possibility of evaluation depends on the availability of reference values as close as possible to 'normal' values of the various blood components considered as reliable descriptors of healthy fish under natural conditions.

Taking into account the long evolutionary history of bony fishes and the many adaptations to different environments, no species can be used as a representative model for all fish. This explains the rapidly expanding literature on the chemical properties of fish blood. However, at present, few reports deal with the blood of primitive fish (e.g. the chondrosteans), as is evident from the extensive reviews of the subject of fish blood [9].

The Adriatic sturgeon, *Acipenser naccarii*, is a species endemic to the waters of North Italy and the countries bordering the eastern Adriatic. Little is known about the ecology and biology of this primitive bony fish [3,11–14,16]. It is generally considered a euryhaline species, because it is sometimes found in estuaries. Once abundant in all the rivers flowing into the North Adriatic Sea, its present distribution range has been greatly reduced by the impact of overfishing and habitat deterioration. Interest in this species was recently aroused by successful attempts at artificial reproduction [1], which suggest that *Acipenser naccarii* may be suitable for fish farming and restocking. Therefore it is important to provide reference values for an assessment of the status of this species in both natural and rearing conditions.

In this initial study on the blood chemistry of captive Italian sturgeons, the concentrations of some components of the serum were measured and the variations resulting from temperature, anaesthesia, crowding and handling, which represent some conditions associated

* Corresponding author. Fax: +39 6 2026189; e-mail: cataldi@uniroma2.it

1095-6433/98/$ - see front matter
PII S1095-6433(98)10134-4

with culture practices and sampling procedure, were recorded.

2. Materials and methods

2.1. Origin and maintenance of fish

Adriatic sturgeon juveniles, derived from artificial reproduction, were reared in the Laboratory of Experimental Ecology and Aquaculture of the University 'Tor Vergata' of Rome. Fish were maintained in square fiberglass tanks supplied with freshwater at 25°C and at a density of 8 kg m^{-3}. Sturgeon were fed on commercial dry pellets (Universal Mangimi).

A group of 12 4-year-old fish, which had mean ($\pm$S.D.) weight and total length of 2.3 ± 1.2 kg and 82.2 ± 13.4 cm, respectively, were used and repeatedly sampled in different conditions, after allowing the specimens to recover for a minimum of 15 days [9]. Fish were fasted for 2 days before sampling.

2.2. Sampling conditions

In the first trial, blood samples were rapidly collected by cardiac puncture from sturgeons serially netted and manually immobilized without anaesthesia.

After 15 days, the recovered sturgeons were sampled after anaesthesia in 140 mg l^{-1} buffered solution of tricaine methane sulphonate (MS 222), which induced loss of body and opercular movements within 4–8 min after exposure.

In the third trial, fish previously acclimated for 1 month to 17°C were sampled after anaesthesia as above.

The sturgeons maintained at a low temperature (17°C) were then exposed to crowding stress by confinement to a tank for 24 h at a density of 16 kg m^{-3}. In addition, the sturgeons were stressed by prolonged handling. Anaesthetized fish were sampled as above.

2.3. Variables examined and analytical methods

Clotted blood was centrifuged at $3000 \times g$ for 10 min and the analyses were performed immediately. Serum osmolality was determined by a cryoscopic method (One-ten Osmometer, Fiske). Na^+, K^+ and Cl^- concentrations were measured using an electrolyte analyzer with ion-specific electrodes (SPOTCHEM SE-1510, Menarini). Glucose, calcium and total protein were determined using an autodry chemistry analyzer (SPOTCHEM SP-4410, Menarini).

Cortisol concentration was measured using Coat-A-Co Cortisol (Kontron Analytical MDA 312) and radioimmunoassay software. The kits were supplied by Diagnostic Product Corporation.

2.4. Data analysis

Range values, means and standard deviations were determined for all the parameters examined. Differences between groups as regards both serum component concentrations and Na:Cl ratio were analyzed by the Mann–Whitney *U*-test. Significance was accepted at $P < 0.01$.

The contribution of Na^+ and Cl^- to total serum osmolality was obtained using the formula $Osm_{NaCl} = (Na^+ + Cl^-) \times 0.91$ [9].

3. Results

The effects of anaesthesia, temperature, crowding and handling stress on mean value of the blood parameters are reported in Table 1.

In all the different sampling conditions tested, the individual plasma cortisol and glucose concentrations were found to be highly variable (as indicated by the range reported in Table 1) and the increase in levels did not depend on the order in which the fish were taken from the tank. The values of these parameters, for example, in sturgeons maintained at 25°C and serially sampled without anaesthesia were, progressively, 45.5, 116.4, 43.0, 77.5, 303.5, 177.1, 17.1, 31.4, 44.7, 186.7, 142.3 nmol l^{-1} and 1.7, 1.8, 2.3, 1.9, 3.9, 3.8, 1.1, 1.6, 1.7, 1.4, 2.2 mmol l^{-1}, respectively. The values of the other parameters were more constant.

The comparison between sturgeon maintained at 25°C sampled with and without anaesthesia showed that the anaesthetized sturgeon differed significantly only in its higher osmolality. The trend of Na^+ and Cl^- concentrations was also towards higher levels, and in fact contributed to total osmolality about in the same percentage ($Osm_{NaCl} = 237.0$ mOsm kg^{-1}; 83.4%) as non-anaesthetized sturgeon ($Osm_{NaCl} = 230.6$ mOsm kg^{-1}; 88%). The Na:Cl ratios in anaesthetized and non-anaesthetized sturgeons, respectively 1.22 and 1.24, were not significantly different.

After acclimation to the temperature of 17°C, the cortisol level and Cl^- concentration decreased significantly. Na^+ and Cl^- ($Osm_{NaCl} = 220.8$) contributed only 79.6% to total serum osmolality, due to very low Cl^- concentration. In fact, the Na:Cl ratio measured 1.46 in this group. The other blood parameters were unaffected by low temperature acclimation.

Crowding and prolonged handling did not seem to affect sturgeon blood chemistry. In fact, no significant increase in mean serum cortisol or glucose levels or in other components were detected. Nevertheless, the individual concentrations of all parameters resulted in a much broader range than the respective values measured in unstressed sturgeon.

Table 1
Mean ± S.D. and range (in parentheses) values of serum constituents of Adriatic sturgeon sampled at 25°C with and without anaesthesia, and at 17°C with and without stress

	Group			
	Not anaethetized, not stressed, 25°C ($n = 11$)	Anaesthetized, not stressed, 25°C ($n = 11$)	Anaesthetized, not stressed, 17°C ($n = 12$)	Anaesthetized, stressed, 17°C ($n = 12$)
Cortisol (nmol l^{-1})	107.8 ± 88.0 (17.1–303.5)	83.6 ± 45.52 (62.2–153.9)	32.0 ± 18.7[b] (13.2–81.4)	43.0 ± 43.8 (2.7–172.1)
Glucose (mmol l^{-1})	2.1 ± 0.9 (1.1–3.9)	2.3 ± 0.9 (1.3–4.0)	2.6 ± 1.1 (1.4–4.6)	3.4 ± 1.6 (1.3–6.7)
Osmolality (mOsm kg^{-1})	262.4. ± 6.9 (251.0–280.3)	273.3 ± 6.0[a] (266.0–286.2)	277.1 ± 3.9 (270.7–283.3)	279.5 ± 6.8 (269.6–291.0)
Na^+ (mEq l^{-1})	140.6 ± 4.8 (134.0–152.0)	143.5 ± 5.2 (136.0–154.0)	144.1 ± 3.7 (137.0–148.0)	143.6 ± 5.5 (135.0–155.0)
K^+ (mEq l^{-1})	3.6 ± 0.3 (3.0–4.3)	3.1 ± 0.3[a] (2.7–3.7)	3.4 ± 0.8 (2.4–5.3)	3.2 ± 0.5 (2.7–4.5)
Cl^- (mEq l^{-1})	112.9 ± 3.5 (107.0–120.0)	117.0 ± 3.1 (112.0–122.0)	98.5 ± 6.5[b] (88.0–107.0)	104.3 ± 3.7 (98.0–111.0)
Ca^{2+} (mEq l^{-1})	2.3 ± 0.1 (2.1–2.6)	2.3 ± 0.3 (1.7–2.8)	2.6 ± 0.3 (1.9–2.9)	2.4 ± 0.3 (1.8–2.9)
T-protein (g dl^{-1})	2.2 ± 0.3 (1.9–2.6)	2.3 ± 0.3 (1.9–2.8)	2.5 ± 0.5 (1.9–3.6)	2.5 ± 0.6 (1.9–3.6)

The four groups were compared using the Mann–Whitney U-test.
[a] Significant difference, at $P < 0.01$, between sturgeon sampled at 25°C with and without anaesthesia.
[b] Significant difference, at $P < 0.01$, between sturgeon sampled at 25°C and 17°C and anaesthetized.

4. Discussion

In sturgeon [7], as well as in teleosts [9], the increase of serum cortisol level was considered a primary indicator of stress response. The other parameters were also liable to change in response to stress or environmental variations.

The serum cortisol levels detected in sturgeon maintained at 17°C are consistent with the 'normal' values reported in *Acipenser baeri*, whereas the high levels found at 25°C are consistent with those found under acute hypoxic stress in the same species [7].

Therefore the cortisol level detected at 17°C might suggest a relatively 'unstressed' condition of captive Adriatic sturgeon at such temperatures, and all the parameters measured might also be considered as 'normal' values. The glucose mean value is lower than resting levels detected in *A. baeri* [7]. Total protein is in the range of values measured in the Russian sturgeon [15]. However, osmolality and ion concentrations might be affected slightly by anaesthesia, as in sturgeons sampled at 25°C. The effect of anaesthesia on salt and water balance was clearly demonstrated in teleosts [4]. Nevertheless, the serum osmolality and ion concentrations measured in the low-temperature group are comparable to the values indicated for *Acipenser transmontanus*, a euryhaline sturgeon [10]. In *A. naccarii* Na^+ and Cl^- contribute to over 75% of total osmolality as in teleosts, whereas the Na:Cl ratio (1.46) may be considered exceptional, and is the same only in anguillids. In other teleosts it averages 1.1 [9].

To elucidate true resting levels of serum parameters of this primitive bony fish, daily and seasonal fluctuations will have to be checked. Moreover, in euryhaline anadromous teleost species, and presumably in the Adriatic sturgeon, some parameters can be linked to physiological adjustments to migration.

The higher cortisol value measured in sturgeon maintained at 25°C may indicate that upper temperature causes chronic stress, which also affects ion concentration. The Adriatic sturgeon was successfully reared at constant high temperature conditions (23 ± 2°C) which offered better growth performance if compared to sturgeon grown both at 15–18°C and at fluctuating temperature regimes (10–30°C) [2]. However, in some teleosts, temperature preferences have been reported and they may depend on endogenous and environmental conditions [5,6].

A. naccarii does not seem susceptible to crowding and handling stress. The mean values of cortisol, glucose and osmolality, in fact, were not altered; furthermore, no apparent cumulative effects were observed. This could suggest the domesticated conditions of the hatchery-reared sturgeons, as it has been demonstrated in chinook salmon [8].

However, the different individual susceptibility to stressors, as indicated by the broad range of values detected, may be an important consideration in the evaluation of certain situations.

Acknowledgements

This research was carried out within the 'Project for the Development of National Aquaculture, 1993–1997' supported by a grant from Italian Ministry of Agriculture (L.201/91).

References

[1] Arlati G, Bronzi P, Colombo L, Giovannini G. Induzione della riproduzione dello storione italiano (*Acipenser naccarii*) allevato in cattività. Riv Ital Acquacolt 1988;23:94–6.

[2] Arlati G, Bronzi P. Sturgeon farming in Italy. In: Gershanovich AD, Smith TIJ, editors. Proceedings of Second International Symposium on Sturgeons, 6–11 September 1993, Moscow. Moscow: VNIRO, 1995:321–32.

[3] Cataldi E, Ciccotti E, Di Marco P, Di Santo O, Bronzi P, Cataudella S. Acclimation trials of juvenile Italian sturgeon to different salinities: morpho-physiological descriptors. J Fish Biol 1995;47:609–18.

[4] Eddy FB. Effects of stress on osmotic and ionic regulation in fish. In: Pickering AD, editor. Stress and Fish. New York: Academic Press, 1981:209–245.

[5] Elliot JM. Some aspects of thermal stress on freshwater teleosts. In: Pickering AD, editor. Stress and Fish. New York: Academic Press, 1981:77–102.

[6] Hazel JR. Thermal biology. In: Evans DH, editor. The Physiology of Fishes. London: CRC Press, 1993:427–467.

[7] Maxime V, Nonnotte G, Peyraud C, Williot P, Truchot JP. Circulatory and respiratory effects of an hypoxic stress in the Siberian sturgeon. Resp Physiol 1995;100:203–12.

[8] Mazur CF, Iwama GK. Effect of handling and stocking density on hematocrit, plasma cortisol, and survival in wild and hatchery-reared chinnok salmon (*Oncorhynchus tshawytscha*). Aquaculture 1993;112:291–9.

[9] McDonald DG, Milligan CL. Chemical properties of the blood. In: Hoar WS, Randall DJ, Farrell AP, editors. Fish Physiology, vol. XIIB. London: Academic Press, 1992:56–133.

[10] McEnroe A, Cech JJ. Osmoregulation in white sturgeon: life history aspects. Am Fish Soc Symp 1987;1:191–6.

[11] Paccagnella B. Osservazioni sulla biologia degli storioni del Bacino Padano. Arch Oceanogr Limnol 1948;5:141–54.

[12] Potts WT, Rudy PP. Aspects of osmotic and ionic regulation in the sturgeon. J Exp Biol 1972;56:703–15.

[13] Rochard E, Castelnaud G, Lepage M. Sturgeons (Pisces: Acipenseridae): threats and prospects. J Fish Biol 1991;37(Suppl. A):123–32.

[14] Rossi R, Grandi G, Trisolini R, Franzoi P, Carrieri A, Dezfuli BS, Vecchietti E. Osservazioni sulla biologia e la pesca dello storione cobice *Acipenser naccarii* Bonaparte nella parte terminale del fiume Pò. Atti Soc Ital Sci Nat Museo Civico Storia Nat Milano 1992;132:121–42.

[15] Skelukhin GK, Metallov GF, Geraskin PP. Effect of temperature and salinity of Caspian sea water on juvenile Russian sturgeon, *Acipenser guldenstadti*. Voprosy Iktiologii 1990;30: 296–304.

[16] Tortonese E. *Acipenser naccarii* Bonaparte, 1836. In: Holcik J, editor. The Freshwater Fishes of Europe. General Introduction to Fishes. Acipenseriformes, vol. I, part II. Wiesbaden: AULA-Verlag, 1989:285–93.