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Changes in serum biochemical parameters of *Acipenser sinensis*, Gray 1835, caused by decreasing environmental salinity

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Summary

Blood serum parameters from Chinese sturgeons (*Acipenser sinensis*) were examined during a 27 day gradual acclimation period from seawater (26.7 salinity) to slightly brackish water (2.5‰) and subsequent transfer to freshwater (0‰ salinity) for a further 30 day period. The results were compared against serum samples from a control group reared throughout in freshwater. For each fish, the levels of 24 serum biochemical parameters were examined. The results indicated that gradual transfer from seawater to brackish water caused the serum concentrations for potassium, sodium, and calcium ions to decrease. This trend was also observed for blood urea-nitrogen (BUN), inosine (CR), uric acid (UA), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), and total bile acid (TBA). All other serum parameters showed only transient changes or no changes at all. After being held subsequently for 30 days in freshwater, the four ion concentrations returned to values considered normal for seawater, indicating that Chinese sturgeon are capable of homeostasis. However, the other six parameters did not return to initial values. In fact, ALP, ALT and Fe^{2+} levels were raised in comparison to the freshwater control group. These observations indicate that major physiological changes occur during the acclimation process. Specifically, at 21‰ salinity a number of biochemical parameters were subject to noticeable fluctuations, suggesting this level may represent the pivotal condition of physiological regulation. Hence, it may be energetically costly for osmoregulatory processes to return to normal in Chinese sturgeon, due to metabolic and nutrient related functions taking much longer to recover.

Introduction

All sturgeon species have complex life histories (Kynard, 1997), requiring tolerance to a large range of environmental conditions at different times of the life cycle, which may be facilitated by physiological mechanisms (Burggren and Randall, 1978). As a result, physiological research on sturgeon has primarily been focused on osmoregulatory capacity (Krayushkina, 1998; Lebreton and Beamish, 1998) and on respiratory parameters (Maxime et al., 1995; McKenzie et al., 1997). Blood parameters have also been used to monitor the effects of water quality and other environmental parameters (Asadi et al., 2006a,b).

The Chinese sturgeon is a typical anadromous fish, which migrates upstream to spawn in the Yangtze River and downstream of the Jinsha Jiang River (Chen, 2007). Due to the construction of the Gezhouba Dam on the Yangtze River, the alternative natural spawning grounds below Gezhouba Dam have been adopted by the species, however the new grounds have also been critically reduced. Furthermore, the geo-topographic environment and the water quality of these new locations are different from the historical spawning grounds (Wei et al., 2005), which may impact on natural reproductive parameters and on the successful recruitment of larvae into the recruiting population, further endangering the survival of this species. Therefore, catching adults already at the estuary and bringing them into a captive environment for propagation before the fish has started to go upstream on its spawning migration, may be an effective measure to allow sturgeons to fully spawn. Moreover, wild sturgeons that are accidentally caught and injured in the estuary require rehabilitation in artificial environments, which are usually located in freshwater. While the serum biochemistry of juvenile Chinese sturgeon cultured in fresh water has been described by Zheng and Li (2007), the impact of changes in environmental salinity on serum biochemical parameters has not been examined in specimens caught in the wild.

This study had, therefore, two major objectives: (i) to provide baseline data on serum biochemical parameters, and (ii) to assess how serum biochemistry is modified following salinity stress. The response of sturgeon serum biochemistry to environmental conditions may be used to evaluate the overall health and nutrition status, as well as the tolerance to environmental change. Consequently, the conditions to maintain the optimal physiological state of this species may be delineated for application to management schemes in captive breeding/rehabilitation centers and may help to understand how optimal sites for reintroduction to natural habitats can be selected.

Materials and methods

Study animals

The fish selected for this experiment were from a 3 year old filial generation. The fish were raised in freshwater holding tanks at the Beijing Aquarium from 2 months onward to year class two. Thereafter, for a 1 year period prior to the present study, a proportion of the raised individuals were transferred to a holding tank containing artificial seawater (after gradual acclimation; i.e. 3% increase in salinity per 3 days over a 30 day period), while the remainder of the cultured population were transferred to the Chinese sturgeon public aquarium, and

raised in fresh water also for a full year. Prior to the start of the trials, the average fork lengths and average weights of experimental sturgeon (n = 6) raised in artificial seawater was 84.3 ± 2.4 cm and 5.1 ± 0.4 kg, respectively. The control group (n = 6) raised in freshwater had at that time an average fork length and average weight of 110.3 ± 2.9 cm and 10.3 ± 0.5 kg, respectively.

Experimental tanks

The seawater holding tank is circular and of dimensions 10 m (diameter) × 0.9 m (height), with a maximum water volume of 72 m^3. The freshwater display aquarium is rectangular and of dimensions 29.0 m (length) × 11.0 m (width) × 4.4 m (height), with a maximum water volume of 1200 m^3. It has a public exhibition window of 20.0 m (length) × 3.0 m (width). The bottom of the aquarium is covered by sand, and the edges of some rocks are covered with soft, non-toxin synthetic resin material to protect the fish from accidental injury. The aquarium is equipped with an advanced life support system, which controls the water quality artificially. The stocking density of the seawater and freshwater tanks are 2.2 and 2.12 kg m^{-3} respectively.

Experimental design

The experiment included two sturgeon groups: (i) a group continuously reared in fresh water, (ii) a group subjected to gradual acclimation from full-strength salinity to freshwater. Before the start of the trials, tests were run to detect any variation in biochemical blood parameters in fish of different size ranges (5–11 kg). No significant differences were found for any of the indicators. As a result 12 fish were selected at random for the two experimental groups.

The experiment on salinity acclimation was run for 57 days. During the first 27 days, the salinity levels were progressively reduced by about 3‰ (mean: 3.0; range: 2.4–3.9; SD: ±0.7) every 3 days (equal amount of freshwater added to the control). Hence, the gradual reduction from the initial salinity level at 26.7‰, passed steps at 24.1, 21.3, 17.5, 13.6, 9.8, 7.4, 5.0, and 2.5 salinity. After a further 3 days, the acclimated sturgeons were transferred to the Chinese sturgeon public aquarium for a further 30 days acclimation to full freshwater.

Blood was sampled from the caudal vein of each fish at 9 days intervals, when the salinity reached 26.7‰, 21.3, 9.8, 2.5, and at thirty days thereafter at 0‰. While originally there were six fish, the freshwater samples are based on four fish only, due to the markers being lost on two fish after transfer to the aquarium tank. The same fish were used throughout the study. Blood was also sampled from the fish in the control group (n = 6) on the same days as from the acclimation group (at 0‰). Prior to the onset of the study, validation experiments had been conducted to confirm that repeated use of syringes on the same animal would not alter the serum biochemical composition being measured (i.e. stress effects) (Yang et al., 2005). In order to minimize the dietary influence on the metabolic status, fish were not fed on the day before blood sampling (Kiffer and Tufts, 1998; Baker et al., 2005). The fish were removed rapidly and placed into a barrel where the individuals were anaesthetized with MS-222 (90 ppm). Blood was drawn via the caudal vessels using a heparin-coated needle and syringe (5 ml).

All biochemical parameters were measured using test kit preparations (SCKISUI, Sekisui Medical Co. Ltd, Kyoto, Japan). All samples were analyzed using an automated biochemical analyzer (OLYMPUS AU400). Serum biochemical parameters determined included: total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL), total protein (TP), albumin (ALB), globulin (GLB), the albumin and globulin ratio (A / G), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), total bile acid (TBA), cholinesterase (CHE), potassium (K^+), sodium (Na^+), chlorine (Cl^-), calcium (Ca^{2+}), phosphorus (P^{3+}), magnesium (Mg^{2+}), iron (Fe^{2+}), blood urea nitrogeon (BUN), inosine (CR), uric acid (UA), total cholesterol (CHO) and glucose (GLU).

Water quality was monitored daily. Temperature (T) and pH values were test using the HANNA convenient pH instrument (combined pH electrode, ATC with automatic temperature compensation, made in Italy). Oxygen (O_2) levels were measured by an infiltration membrane technique using HACH (made in America), NH_3 / NH_4^+ and NO_2^- were measured by HACH Spectrophotometer (made in America). Water quality was maintained under the following conditions: T: 21–24°C, O_2: 7–10 mg L^{-1}, pH: 7.2–8.0, NH_3 / NH_4^+: 0.01–0.05 mg L^{-1}, NO_2^-: 0–0.1 mg L^{-1}.

Data analyses

For all measurements, both treatment groups were analyzed for normality using Normality plots with tests. To examine whether there is any significant difference at each salinity level, species and treatment effects for each variable, parameters were assessed for the biochemical variables using a two-way ANOVA test. Duncan's test was also used to determine where differences occurred. Independent-Sample *T* Tests were used to determine if there were differences between the treatment group and the control group. Where the assumption of normality was violated, ANOVA on ranks (Friedman test) was used to confirm the results. The Mann–Whitney *U* test was used to determine whether there were differences between the treatment group and control group. Alpha (α) in all cases was 5% ($P < 0.05$).

All values are presented as mean ± SE. All statistics were performed using SPSS (ver. 11.0).

Results

Figure 1 presents the change in Na^+, Cl^- ion serum levels in the acclimation group, from sea water (26.7‰) to freshwater across the 57 days experimental period. The decline was both gradual and significant. During the subsequent 30 days period, when fish were maintained in fresh water, both the Na^+ and Cl^- values were observed to rise to that similar to fish in both in seawater (26.7 salinity) and in the control group. The other ions, Ca^{2+}, P^{3+}, Mg^{2+} serum Fe^{2+}, and ALT (Tables 1 and 2), showed a similar pattern of change to Na^+ and Cl^-. The only difference found was for the K^+ value, which showed no significant difference, despite a similar change being recorded. Also, TBA showed a decrease in concentration with declining salinity from 26.7 seawater to 2.5 slightly brackwater, but subsequently high levels were recorded in freshwater, although no significant difference was found.

The results presented in Tables 1 and 2 show no significant changes in TBIL, DBIL, IBIL TP levels during declining salinity. The only exception was TP, when compared with the control group. Also, an increase in AST levels was recorded at the salinity of 21.3, followed by a significant declining trend.

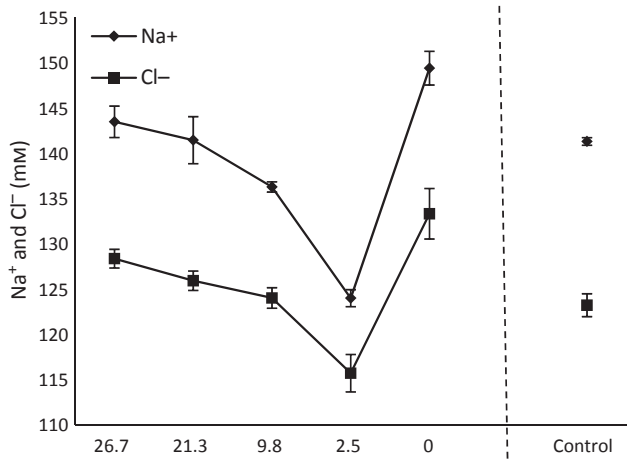

Fig. 1. The Na^+ and Cl^- ion serum levels of sturgeon *Acipenser sinensis* during the 27 day acclimation period from sea water (26.7‰) to slightly brackish water r (2.5‰), and after a 30 day period in freshwater (0‰). The values represent mean ± SE. At each concentration n = 6 fish (the same each time), except for 0‰ salinity when n = 4; control group n = 6 fish

After 1 month of fresh water acclimation, the AST value was lower than that recorded for the control fish. All salinity treatments showed no significant difference when compared to the control group. The change in ALB values followed a similar pattern to AST, although no significant difference was found. All salinity treatments (except for 2.5) showed no significant difference when compared to the control group.

Figure 2 shows that A/G levels fell significantly during acclimation to different salinity levels and during the 30 day period at 0‰ salinity. A significant difference was found for GLU with decreasing salinity. However, at a salinity of 21.3, a sharp decrease was recorded, followed by an immediate increase after 1 month in fresh water ($P < 0.05$). The recorded decrease was also statistically significant (Fig. 3). BUN, CR, UA and ALB values showed a similar pattern of change to A/G. The only differences were that CR, UA and ALB presented no statistical difference during acclimation to decreasing salinity, and UA showed no statistical difference when compared to the control group. In contrast, ALP, and GGT levels changed inversely when compared to GLU, but GGT showed no statistical significance.

There was a significant increase in GLB with decreasing salinity, with the final values being similar to that of the control group (Fig. 4). Thus, towards the end of the trial there was no significant difference for GLB at different salinity levels when compared with the control group (Table 2).

Discussion

In the present study, we found that ion concentrations may return to initial levels, when the sturgeon are moved from seawater to freshwater conditions. This finding indicates that, at least for the Chinese sturgeon, osmoregulatory processes fulfill their function, supporting existing studies of other species of sturgeon, including *A. naccarii* and *A. transmontanus* (McEnroe and Cech, 1985; Martínez-Álvarez et al., 2002). In a previous experiment on *A. fulvescens*, when salinity levels were below 25‰, the serum concentrations of Na^+, K^+, Cl^-, and Ca^{2+} ions were found to increase with increasing salinity (Lebreton and Beamish, 1998). The different rhythms in acclimation recorded between this research and our study may be explained by differences in osomoregulatory ability among different species and even populations of sturgeon. Furthermore, in our study we found significant differences in Cl^-, Ca^{2+} and P^{3+} levels, but not in K^+, Na^+, Mg^{2+} levels,

Table 1
Serum biochemical parameters of *A. sinensis* at different salinity levels. Mean ± SE are presented. Data values with different letters indicate significant differences ($P < 0.05$). Two-way ANOVA test and Duncan's test were used for parameters K^+,Ca^{2+}, P^{3+}, Mg^{2+}, UA, TP, ALB, GLB, A/G, ALT, AST, ALP, CHE, Fe^{2+}. ANOVA on ranks (Friedmen tests) and two-way ANOVA tests were used for Na^+, Cl^-, BUN, CR, TBIL, DBIL, IBIL, GGT, TBA, GLU. At each concentration, n = 6 fish (the same each time), except for 0‰ salinity when n = 4; control group n = 6 fish

	Salinity (‰)					
Parameter	26.73 (n = 6)	21.3 (n = 6)	9.8 (n = 6)	2.5 (n = 6)	0 (n = 4)	0 (Control) (n = 6)
K^+ (mM L^{-1})		3.01 ± 0.15	2.69 ± 0.09	2.51 ± 0.08	2.71 ± 0.21	2.19 ± 0.16
Na^+ (mM L^{-1})	143.47 ± 1.74^{ac}	141.44 ± 2.60^{ac}	136.27 ± 0.56^{ab}	123.98 ± 0.94^{b}	149.40 ± 1.86^{c}	141.30 ± 0.42
Cl^- (mM L^{-1})	128.35 ± 1.03^{ad}	125.91 ± 1.07^{ab}	124.00 ± 1.13^{b}	115.69 ± 2.07^{c}	133.31 ± 2.79^{d}	123.20 ± 1.26
Ca^{2+} (mM L^{-1})	1.88 ± 0.05^{a}	1.86 ± 0.05^{ab}	1.77 ± 0.03^{bc}	1.57 ± 0.01^{d}	1.73 ± 0.09^{c}	1.71 ± 0.02
P^{3+} (mM L^{-1})	2.93 ± 0.08^{a}	3.60 ± 0.23^{b}	2.84 ± 0.07^{a}	2.24 ± 0.03^{c}	2.83 ± 0.18^{a}	2.64 ± 0.07
Mg^{2+} (mM L^{-1})	0.99 ± 0.06^{ab}	1.08 ± 0.04^{b}	0.93 ± 0.03^{a}	0.81 ± 0.02^{c}	0.98 ± 0.09^{ab}	0.98 ± 0.01
BUN (mM L^{-1})	3.42 ± 0.40^{a}	2.77 ± 0.28^{a}	1.67 ± 0.48^{b}	1.75 ± 0.28^{b}	0.75 ± 0.21^{c}	0.42 ± 0.11
CR (μM L^{-1})	9.20 ± 1.13	6.57 ± 1.01	7.78 ± 0.89	4.12 ± 1.50	5.38 ± 1.48	4.53 ± 0.08
UA (mM L^{-1})	2.67 ± 1.40	0.80 ± 0.47	2.68 ± 1.14	1.13 ± 0.47	1.98 ± 0.62	1.75 ± 0.93
TBIL (μM L^{-1})	0.23 ± 0.03	0.17 ± 0.02	0.20 ± 0.03	0.18 ± 0.02	0.35 ± 0.13	0.33 ± 0.03
DBIL (μM L^{-1})	0.10 ± 0.02	0.08 ± 0.02	0.15 ± 0.02	0.12 ± 0.02	0.08 ± 0.03	0.22 ± 0.03
IBIL (μM L^{-1})	0.13 ± 0.04	0.08 ± 0.02	0.05 ± 0.03	0.07 ± 0.02	0.28 ± 0.11	0.12 ± 0.01
TP (g L^{-1})	16.12 ± 1.53	18.32 ± 1.04	17.63 ± 1.50	17.43 ± 0.98	17.88 ± 2.38	18.72 ± 1.03
ALB (g L^{-1})	4.53 ± 0.52	4.88 ± 0.41	4.47 ± 0.47	4.14 ± 0.35	3.95 ± 0.78	5.32 ± 0.41
GLB (g L^{-1})	11.58 ± 0.98^{a}	13.43 ± 0.65^{ab}	13.17 ± 1.05^{ab}	13.25 ± 0.64^{ab}	13.93 ± 1.62^{bc}	13.40 ± 0.67
A/G	0.39 ± 0.02^{a}	0.36 ± 0.02^{b}	0.34 ± 0.01^{c}	0.31 ± 0.01^{d}	0.28 ± 0.03^{e}	0.40 ± 0.02
ALT (U L^{-1})	56.70 ± 16.01^{a}	40.47 ± 5.48^{ab}	25.93 ± 3.43^{b}	23.63 ± 2.56^{b}	34.65 ± 4.65^{b}	15.55 ± 0.69
AST (U L^{-1})	142.33 ± 7.21^{a}	234.03 ± 33.89^{b}	188.40 ± 14.16^{ab}	186.43 ± 18.35^{ab}	152.68 ± 24.91^{a}	163.22 ± 11.96
ALP (U L^{-1})	127.17 ± 5.65^{a}	989.83 ± 112.22^{b}	568.58 ± 68.31^{c}	450.67 ± 46.67^{c}	928.55 ± 122.42^{b}	508.48 ± 31.43
GGT (U L^{-1})	0.40 ± 0.16	4.87 ± 2.80	0.65 ± 0.07	0.20 ± 0.03	31.88 ± 16.51	2.32 ± 1.34
TBA (μM L^{-1})	0.73 ± 0.09	0.72 ± 0.11	0.54 ± 0.05	0.45 ± 0.03	1.10 ± 0.47	0.50 ± 0.08
Fe^{2+} (mM L^{-1})		2.21 ± 0.61^{a}	2.68 ± 0.44^{ab}	1.22 ± 0.30^{ac}	5.03 ± 0.60^{d}	4.5 ± 0.66
GLU (mM L^{-1})	1.82 ± 0.23^{a}	0.13 ± 0.03^{b}	0.93 ± 0.12^{c}	1.28 ± 0.09^{a}	0.63 ± 0.30^{c}	1.20 ± 0.10

Table 2
Showing the significant differences of serum biochemical parameters between the value for each salinity level and the control group. 'a' indicates significant difference ($P < 0.05$). NS indicates no significant difference. Independent-sample *T* Tests were used for parameters K^+, Ca^{2+}, P^{3+}, Mg^{2+}, UA, TP, ALB, GLB, A/G, ALT, AST, ALP, CHE, Fe^{2+}. Mann–Whitney *U* test were used for Na^+, Cl^-, BUN, CR, TBIL, DBIL, IBIL, GGT, TBA, GLU. At each concentration n = 6 fish (the same each time), except for 0‰ salinity when n = 4; control group n = 6 fish

	Salinity (‰)				
Parameter	26.73 (n = 6)	21.3 (n = 6)	9.8 (n = 6)	2.5 (n = 6)	0 (n = 4)
K^+ (mM L^{-1})	NS	a	a	NS	NS
Na^+ (mM L^{-1})	NS	NS	a	a	NS
Cl^- (mM L^{-1})	a	NS	NS	a	a
Ca^{2+} (mM L^{-1})	a	a	NS	a	NS
P^{3+} (mM L^{-1})	a	a	a	a	NS
Mg^{2+} (mM L^{-1})	NS	NS	NS	a	NS
BUN (mM L^{-1})	a	a	a	a	NS
CR (μM L^{-1})	a	NS	NS	NS	NS
UA (mM L^{-1})	NS	NS	NS	NS	NS
TBIL (μM L^{-1})	NS	a	a	a	NS
DBIL (μM L^{-1})	a	a	NS	a	a
IBIL (μM L^{-1})	NS	NS	NS	NS	a
TP (g L^{-1})	NS	NS	NS	NS	NS
ALB (g L^{-1})	NS	NS	NS	a	NS
GLB (g L^{-1})	NS	NS	NS	NS	NS
A/G	NS	NS	a	a	a
ALT (U L^{-1})	a	a	a	a	a
AST (U L^{-1})	NS	NS	NS	NS	NS
ALP (U L^{-1})	a	a	NS	NS	a
GGT (U L^{-1})	NS	NS	NS	NS	NS
TBA (μM L^{-1})	NS	NS	NS	NS	NS
CHE (U L^{-1})	NS	a	a	a	NS
Fe^{2+} (mM L^{-1})	NS	a	a	a	NS
GLU (mM L^{-1})	NS	a	NS	NS	NS

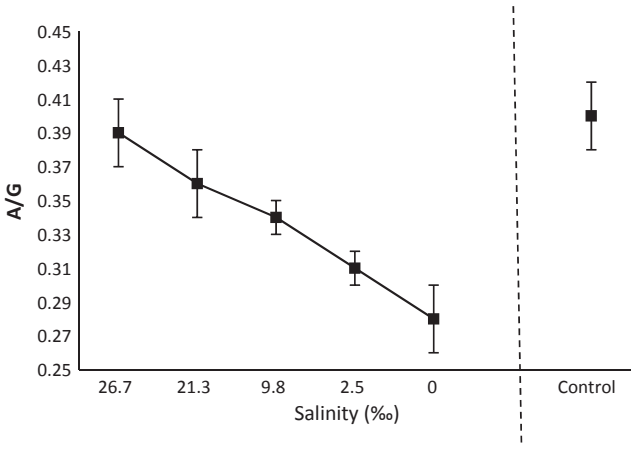

Fig. 2. The ratio of albumin and globulin (A : G) levels of sturgeon *Acipenser sinensis* during the 27 day acclimation period from sea water (26.7‰) to slightly brackish water r (2.5‰), and after a 30 day period in freshwater (0‰). The values represent mean ± SE. At each concentration n = 6 fish (the same each time), except for 0‰ salinity when n = 4; control group n = 6 fish

when comparing the seawater group (26.7‰) with the freshwater controls. This observation indicates that a difference exists in the ion levels between fish acclimated to seawater vs fish acclimated to freshwater. The study by McEnroe and Cech (1985) on freshwater *Acipenser transmontanus* indicated that concentrations of Cl^- in the seawater group were greater than in the freshwater group. In contrast, the values of K^+ and

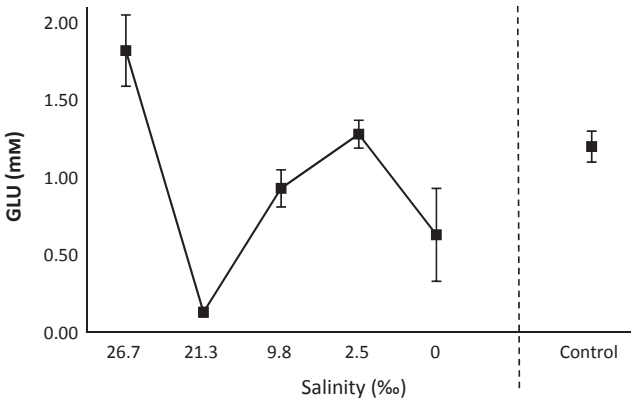

Fig. 3. Glucose (GLU) values in the serum of sturgeon *Acipenser sinensis* during the 27 day acclimation period from sea water (26.7‰) to slightly brackish water r (2.5‰), and after a 30 day period in freshwater (0‰).The values represent mean ± SE. At each concentration n = 6 fish (the same each time), except for 0‰ salinity when n = 4; control group n = 6 fish

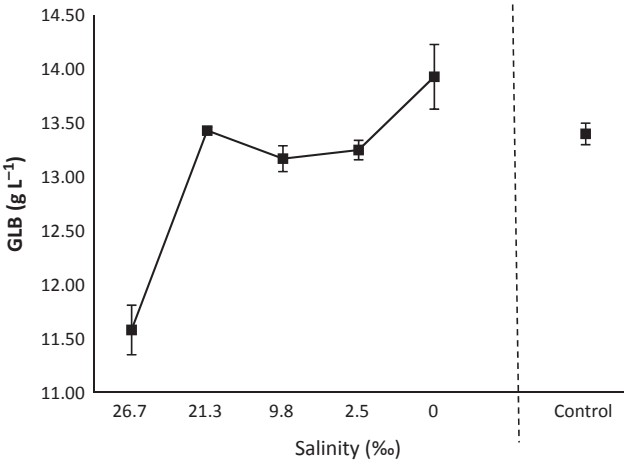

Fig. 4. Globulin (GLB) levels in the serum of sturgeon *Acipenser sinensis* during the 27 day acclimation period from sea water (26.7‰) to slightly brackish water r (2.5‰), and after a 30 day period in freshwater (0‰). The values represent mean ± SE. At each concentration n = 6 fish (the same each time), except for 0‰ salinity when n = 4; control group n = 6 fish

Na^+ were found to be similar between both groups. In another study by Natochin et al. (1985), it was found that serum ion concentrations were similar between seawater and freshwater groups. This finding would account for the different serum ion concentrations that were recorded during fish acclimation in our study, both from seawater to freshwater, and during the prolonged period in freshwater.

The epithelium of the gill have a great permeability to the K^+, Na^+, Cl^-, Ca^{2+}, and excessive mono-ion was secreted through the gill in seawater teleosts, while divalent and trivalent ions are secreted in the form of urine (Lin, 1999). Hence, the kidney may play an important role in the regulation of P^{3+} and Mg^{2+} concentrations. In our study, P^{3+} and Mg^{2+} concentrations increased noticeably at a salinity of 21.3‰. This sudden rise may be explained by a major change in kidney function at this specific salinity level. In other words, the structure of the kidney may have been modified to adapt the alteration in salinity (Krayushkina et al., 1996).

CR, BUN, and UA comprised the main metabolic products that were excreted through the gills and kidney. The final products of protein in fish are CR, BUN, UA, NH_3 and

TMAO etc. In freshwater teleosts, most nitrogenous waste (NH_3/NH_4^+) is discharged via the gill, while in marine teleosts, a large proportion of nitrogenous waste (TMAO) is discharged in the urine (Lin, 1999). In the present study, the decreasing trend of CR, BUN and UA with declining salinity in *A. sinensis* may indicate that, for this euryhaline species, when the chemical receptor in the gills sense an alteration in water salinity (Zhao et al., 2006), the type of nitrogenous waste and mode of excretion (i.e. via gills or in urine) may be adjusted in response to the switch from seawater to freshwater. As a result, there is an increase in the proportion of ammonia that is discharged from the gills. Thus, in the current study, the proportion of NH_3 increased with respect to total nitrogenous waste, while CR, BUN and UA decreased.

Our results indicated that, in *A. sinensis*, salinity acted as a stressor promoting the alteration of liver function. The transfer of the fish from seawater to brackish water caused a major change in the concentrations of TBA, ALT, A/G, GLB, AST, ALP, and GGT. Existing research has shown that the concentration of TP, ALB, GLB, A/G, TBA, ALT, AST, ALP, and GGT in plasma serve as indicators of liver function (Berneta et al., 2001). Changes in the levels of these parameters may explain the hypofunctioning of the liver cells under changing salinity levels. TP includes both ALB and GLB. GLB is related to immunity (Lin, 1999), the levels of which increase in the presence of infection or injury (Chen and Guan, 1987). In addition, in the present study, A/G levels continued to decrease after transferring the fish into the fresh water aquarium, which demonstrated that salinity variation and environment alteration act as stressors that boost body immunity. However, the effect of the duration and intensity of such stressors on the immunity of Chinese sturgeon requires further investigation, as it would indicate the species capacity to endure new environmental conditions and hence long-term survival.

There are a number of studies demonstrating that the reaction of fish to stress includes changes in hormone levels, blood cell parameters, enzyme activity, and other biochemical related processes (Donaldson, 1981; Martínez-Álvarez et al., 2002). Fan et al. (2000) noted a decline in erythrocyte function and a shortening of lifespan in *Sciaenops ocellatus* with decreasing salinity. In comparison, Huang et al. (2006) showed a decrease in erythrocyte counts and increase in MCV levels, however, for *Aclpenser schrenckii* a decline in the total function of erythrocytes did not occur during acclimation from freshwater to saline waters. In the current study, there was an increase in Fe^{2+} with decreasing salinity, which may indicate that erythrocyte count increased as a result of increasing Fe^{2+} serum levels when Chinese sturgeon occupy waters of lower salinity, however, this observation requires confirmation by further study.

It should be emphasized that TBIL, DBIL, CR, UA, and GLU levels decreased while TP, GLB, AST, ALP, GGT, P^{3+} and Mg^{2+} levels increased significantly at a salinity of 21.3‰. This record indicates that the physiology of Chinese sturgeon may be subject to specific regulation. It may also be inferred that a salinity of 20% may represent the limit of physiological regulation in Chinese sturgeon. This observation supports existing research, in which a salinity of 20% has been found to represents the limit of physiological regulation limit in *A. naccarii*, at which point poor growth and high mortality occurred (Cataldi et al., 1995; McKenzie et al., 1999).

Furthermore, Huang et al. (2006) noted that as environmental salinity increased, fish consumed more energy, while glucose and lipids provided the energy required for metabolism. Hence, when the available food source lacks sufficient energy, protein in the feed would be utilized as energy source (Lin, 1999). During this study, there was no alteration in the amount of food consumed by the sturgeon. Furthermore, TP levels remained stable, whereas there was a noticeable decline in GLU levels, both of which indicated that the osmoregulatory process required more energy to respond to these physiological changes.

When the fish remained in the freshwater aquarium for 30 days, GLU levels declined significantly to values lower than the controls, while ALT, ALP, Fe^{2+} levels increased significantly to levels higher than in the control. Sturgeon feeding levels were normal, indicating that the alteration in environmental conditions acted as a temporary stressor to regulate physiological responses while accelerating energy utilization.

In conclusion, when the sturgeon *A. sinensis*, is subjected to a decline in salinity to 2.5‰, a number of physiological responses of the fish occur. For example, serum ion levels and blood parameters related to metabolism and nutrition were affected. This observation indicated that osmotic stress counteracted by osmoregulation is involved in the acclimation of sturgeon to decreased salinities (Martínez-Álvarez et al., 2002). The ion values returned to normal for both seawater and freshwater conditions, after 1 month in salinity acclimation. This finding demonstrated that osmoregulatory processes may fulfill their function after a period of acclimation. However, there was a change in some blood parameters, related to metabolism and nutrition, which did not return to initial seawater and freshwater values. Hence, osmoregulatory processes in sturgeon may cause major alterations to the metabolic process, indicating that extreme change in ion regulation is energetically costly.

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