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Effects of temperature, salinity, illumination and Cu^{2+} on oxygen consumption of juvenile Chinese sturgeon *Acipenser sinensis*

Jianyi Liu[1], Ming Duan[1,2*], Liang Qu[1,3], Longzhen Zhang[1], Guangpeng Feng[1], Tao Zhang[1], Junli Hou[1], Wengang Yan[1], Xiaorong Huang[1], Ping Zhuang[1,3*]

[1]*Key and Open Laboratory of Marine and Estuarine Fisheries Resources and Ecology, Ministry of Agriculture, East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai, 200090, China*
[2]*State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China*
[3]*College of Fisheries and Life Science, Shanghai Ocean University, Shanghai, 201316, China*

Abstract

The oxygen consumption rate (*OCR*) of juvenile Chinese sturgeon *Acipenser sinensis* (Body weight, range 5 - 32 g) were determined under different environmental conditions, including water temperature, salinity, illumination intensity and metal ion concentration [Cu^{2+}] by single factor experimental analysis. *OCR* and opercula frequency (*OF*) increased with rising of water temperature. The linear relationships between water temperature (*WT*) and *OF* with *OCR* can be described as $OCR = 0.018\ WT + 0.016$ ($R^2 = 0.988$, $P < 0.01$), $OCR = 2.737\ OF + 77.726$ ($R^2 = 0.64$, $P < 0.01$), respectively. The acute exposure experiments showed that the *OCR* also increased initially with an increase in salinity. The linear relationships between salinity (*S*) and *OCR* can be described as $OCR = 8.1 \times 10^{-5} S^3 - 3.01 \times 10^{-3} S^2 + 0.0263\ S + 0.2729$ ($R^2 = 0.995$, $P < 0.01$). Three days of acclimation to different salinities, the *OCR*s decreased sharply relative to the first day exposure values. The equation $OCR = - 6.29 \times 10^{-4} S^2 + 0.0238\ S + 0.2797$ ($R^2 = 0.988$, $P < 0.01$) illustrates the linear relationship between salinity and *OCR*s after 3-day exposure. After 3-day acclimation, the *OCR*s increased also with increasing light intensity, except for the ambient light group (i.e., 2000 lx) and the group exposed to direct sunlight (i.e., 20000 lx). After 1-day exposure to different Cu^{2+} concentrations (*C*), the *OCR*s decreased rapidly in all of the groups. But the *OCR* level being gradually different with the increasing of Cu^{2+} concentration, $OCR = 0.237^{e\ -\ 0.09\ C}$ ($R^2 = 0.898$, $P < 0.01$). The results provided us with some useful guide lines which may apply into protection and cultivation management of this endangered species.

Keywords: Chinese sturgeon, *Acipenser sinensis*, Opercula frequency, Oxygen consumption rate, Temperature, Salinity, Illumination, Cu^{2+}

Introduction

The Chinese sturgeon *Acipenser sinensis* is an anadromous protected species that presently spawns only in the Yangtze (Changjiang) River, China. As a result of damming, overfishing, hydro-dam construction and

* Corresponding author. E-mail: fisherduan@gmail.com, pzhuang@online.sh.cn. Tel: +862165807898, Fax: +862165683926.

environmental pollution, populations of sturgeon species have greatly declined since 1983 (Zhuang et al. 2002, 2006), and recruit population quantity of Chinese sturgeon decreased by 80% during 1981 to 1999 (Chang and Cao 1999; Liu et al. 2006c). Commercial capture of Chinese sturgeon was prohibited and in 1988 the species was listed as a Category I protected species (the highest level of protection in China) (Zhuang et al. 2002, 2006). The studies on reproductive biology, controlled propagation and wildlife resource survey of Chinese sturgeon are under way (Yi 1994; Liu et al. 2006a, 2007a, b; Zhu et al. 2006; Yang et al. 2008).

The oxygen consumption rate (*OCR*), which is a comprehensive reflection of various physiological process of fish, is an important factor in metabolism (Fry 1971); and the amount of dissolved oxygen available in culture media plays a key role in the successful hatching of fertilized eggs and larval development as well as the ongrowing of other life cycle stages (Chen et al. 1999). Multiple factors may affect the metabolic rate as well, including developmental stage, physiological state and some environmental factors (Chen et al. 1999). There are numerous researches on *OCR* of many sturgeon species in different life stages, such as Amur sturgeon *A. schrenckii* (Hou et al. 2007), Paddlefish *Polyodon spathula* (Xu et al. 2008) and Chinese sturgeon (Liu et al. 2006b). They provided basic data for productive practice and species conservation of sturgeons.

However, to our knowledge, there has not been any report about the *OCR* of the Chinese sturgeon under different environmental factors. Here, by single factor experimental analysis, the oxygen consumption rate (*OCR*) of juvenile Chinese sturgeon *Acipenser sinensis* were measured under different environmental conditions, including water temperature, salinity, illumination intensity and metal ion concentration [Cu^{2+}]. It will help to understand the metabolic requirements of the species, which are of great significance in understanding the ecology of the species in natural habitats, but also for managing their cultivation.

Materials and methods

Fish and rearing conditions

The juvenile Chinese sturgeon (body weight, rang 5 - 32 g) used in this study were 2 months old which were propagated at the Gezhou dam Chinese sturgeon research institute, Hubei, China. Juvenile individuals were chosen with even size, normal body color and robust physique. They were reared for 15 days in aquaria (diameter 99 cm, height 45 cm) before testing in each experiment.

Tap water was used and the water was filtrated by active carbon (surplus chlorine was lower than 0.01 mg/l), the pH value was 7.6 ± 0.1 and water temperature was 20 ± 1 ºC, whereas the water bath boiler was used to control the water temperature. Salt (Synthetic sea salt produced by Huai'an Tong Bo Co., Ltd, Jiangsu, P. R. China) was used to regulate salinity scale, illumination intensity was modulated using fluorescent lamp, and 200 mg/l Cu^{2+} original solution was confected with $CuSO_4 \cdot 5H_2O$ and chlorin-free pure water. The environmental factors used in the study were temperature, salinity, illumination and Cu^{2+}. The temperature were 5, 10, 20, 25, 26 and 30 ºC, the salinities were 0, 5, 10, 15 and 20, the illumination intensities were 0, 40, 500, 2000 and 20000 lx, and the Cu^{2+} concentrations were 0, 5, 6, 7, 8 and 9 mg/l.

Oxygen uptake experiment

The still-water type respiration chamber method was employed in this study. The test device was a rectangular glass container with a volume of 6 l according the sturgeon sizes, which permitted the juvenile sturgeons to swim freely with the dissolved oxygen higher than 6.0 mg/l during the experiment.

Three replicate groups were set per each trial and one control group was also set in each trial, in which the same water was filled as the experimental group with no fish. After 1 day of starvation, five fish from each group were chosen randomly from the tank and transferred into each chamber described above for 3 - 5 days acclimation respectively. Then the chambers were sealed. Each oxygen consumption trial was conducted at 13:00 and lasted for 2 hours. At the start of the experiment, the air in each respiration chamber was discharged completely and the respiration chambers were put into the water bath to keep constant temperature with the temperature error controlled within ± 0.5 ºC. The oxygen consumption rate (OCR) was monitored after 1 day of acclimation in the Cu^{2+} concentration trial, whereas 3 days of acclimation in the temperature and illumination trials. But in the salinity trial, the OCR was determined under different conditions: (I) immediately after the fish exposed to the salinity solution, and (II) 3 days of acclimation to the salinity environment.

In each trial tank, dissolved oxygen was monitored before and after the test. The trial tank was reversed 3-5 times to reduce the sampling error prior to sampling the water. Water was sampled via a bottom siphon (Dong and

Zhang 1992). Dissolved oxygen was determined by Winkler's iodine measure method (Furuya and Harada 1995). Six water samples were monitored in each group. At the end of the experiment, fish weight was measured (nearest to 0.01 g).

Data analysis

OCR was calculated according to the following equation:

$OCR = (C_1 - C_2) \times V / WT$.

Where C_1 and C_2 represented the amount of dissolved oxygen (mg) in the control and test tanks respectively, V represented the volume (l) of the trial tank, W represented the body weight (g) of the fish, and T represented respiration time (h).

Temperature coefficient Q_{10} showed the ratio of consumption oxygen variation when temperature increased 10 ºC, it calculated according to the following equation:

$Q_{10} = (R_2 / R_1)^{10/(t_2-t_1)}$.

Where t_1 and t_2 represented the temperature of two group trials respectively, R_1 and R_2 represented corresponding OCR under each temperature group. In this experiment, the group of $t_2 - t_1 = 10$ were selected to calculate Q_{10}, thus, $Q_{10} = R_2 / R_1$.

Statistical differences were evaluated using a one-way analysis of variances (ANOVA). Multiple comparisons were carried out using Duncan's method if significant differences were found. For evaluating correlations between two variables, Pearson's correlation analysis was used. Differences were regarded as significant when $P < 0.05$. All the data were analyzed with SPSS software (version 13.0) and described as mean ± standard deviation (SD).

Results

Effects of temperature on OCR

It showed that *OCR* increased with the increase of temperature at similar body weight (Table 1). The linear relationship between *OCR* and temperature (*T*) could be described as $T = 0.018\ OCR + 0.016$. It showed that the relationship between water temperature and *OCR* is highly significant ($R^2 = 0.988$, $P < 0.01$). Opercula frequency increased with the increase of water temperature. The linear relationship between the opercula frequency (*OF*) and temperature (*WT*) could be described as $OF = 2.737\ WT + 77.726$, and the relationship was highly significant ($R^2 = 0.64$, $P < 0.01$). Q_{10} was used to describe the difference oxygen consumption at different temperature ranges. The maximum Q_{10} was 2.2291 when the temperature enhanced from 5 ºC to 15 ºC, and the minimum Q_{10} was 1.4251 when the temperature ranged from 20 ºC to 30 ºC.

Table 1. Mean body weight (*BW*), opercula frequency (*OF*), oxygen consumption rate (*OCR*) and sampling number (N) of the juvenile Chinese sturgeon *Acipenser sinensis* at different water temperatures (*WT*)

WT (ºC)	*BW* (g)	N (ind.)	*OF*[*]	*OCR* (mg/g/h)	Q_{10} Value
5	13.35	15	84.0 ± 8.5[a]	0.124 ± 0.003	-
10	13.11	15	103.7 ± 16.0[b]	0.178 ± 0.004	-
15	13.27	15	122.0 ± 6.7[c]	0.277 ± 0.005	2.23
20	13.27	15	132.3 ± 16.7[c]	0.386 ± 0.010	2.17
25	12.53	15	150.6 ± 13.9[d]	0.497 ± 0.035	1.79
30	11.55	15	153.7 ± 23.4[d]	0.550 ± 0.014	1.43

[*] Means with different letters in the same columns were significantly different ($P < 0.05$).

Relationship between suffocation point and body weight

The dissolved oxygen was monitored when first Chinese sturgeon sacrificed and 80% juveniles died at 20 ºC water temperature, respectively. Suffocation point (*SP*) was defined as dissolved oxygen (mg/l) when 80% experimental fish sacrificed. As the results, *SP* decreased with the increase of the body weight (*BW*) (Table 2). The *SP* of Chinese sturgeon ranged from 1.445 to 1.725, which was higher than that of any other fishes. There was a negative correlation between the *SP* and the body weight of the juvenile Chinese sturgeon in such closed environments, which could be described as $SP = 1.748 - 0.009\ BW$ ($R^2 = 0.964$, $P < 0.01$). The decrease of dissolved oxygen caused intoxication symptoms of juveniles before death, such as increased routine ventilation rates, deepened body color, sometimes hypoexcitability, violent aimless movements with a loss of equilibrium, exanimation, decreased routine ventilation rates, and ultimately leading to sacrifice.

Table 2. Mean body weight (*BW*), water temperature (*WT*), and sampling number (N) of juvenile Chinese sturgeon *Acipenser sinensis* at different dissolved oxygen (DO) concentrations

BW (g)	*WT* (ºC)	N (ind.)	Initial DO (mg/l)	DO at first death (mg/l)	DO at mortality of 80% (mg/l)
5.01	20	5	7.597	1.839	1.725
5.45	20	5	7.597	1.828	1.711
8.75	20	5	7.409	1.798	1.658
10.20	20	5	7.362	1.778	1.613
28.10	20	5	7.597	1.736	1.489
32.35	20	5	7.597	1.705	1.445

Effects of salinity on OCR

Figure 1 shows that the *OCR* of the juveniles was in significantly positive correlation with the salinity. The Chinese sturgeon was transferred to different salinity groups from the fresh water under 15 ºC (body weight, 15.57 ± 0.27 g). The *OCR* decreased with the increase of the salinity. The relationship between *OCR* and salinity *(S)* could be described as $OCR = 8.1 \times 10^{-5}\ S^3 - 3.01 \times 10^{-3}\ S^2 + 0.0263\ S + 0.2729$ ($R^2 = 0.995$, $P < 0.01$).

The *OCR* of the juvenile Chinese sturgeon showed highly significant after exposure to the different salinity for 3 days ($P < 0.01$). The *OCR* increased firstly and then decreased with the increase of salinity. The maximum *OCR* was 0.342 mg/l when the salinity was 5, and the *OCR* began to decrease when the salinity got over 5. The *OCR* was significantly higher than the control group when the salinity was 10 ($P < 0.05$), while it was significantly lower than the control group at salinity 15 and 20 ($P < 0.05$). It reached the lowest value at salinity 20 which decreased by 12.84%, compared with the control group. The relationship between salinity and *OCR* could be described as $OCR = -6.29 \times 10^{-4}\ S^2 + 0.0238\ S + 0.2797$ ($R^2 = 0.988$, $P < 0.01$).

Effects of illumination on OCR

The *OCR*s of juvenile Chinese sturgeon at 4 illumination intensity (water temperature, 20 ºC) were monitored with similar body weight (Table 3). The one-way ANOVA analysis indicated that the *OCR*s were apparently different among all groups ($P < 0.05$) except natural light (2000 lx) group and direct sunlight (20000 lx) group ($P > 0.05$). The *OCR* fell to the nadir in the dark environment and roared to the zenith in direct sunlight environment. In direct sunlight environment it was 1.83 times higher than that in the dark environment. The *OCR*s increased obviously with the increase of illumination intensity. It showed that the illumination intensity significantly affected the metabolic activity of the juvenile individuals, which may have some relationship with increase of activity of foraging for food, avoiding predators and other activities under direct sunlight.

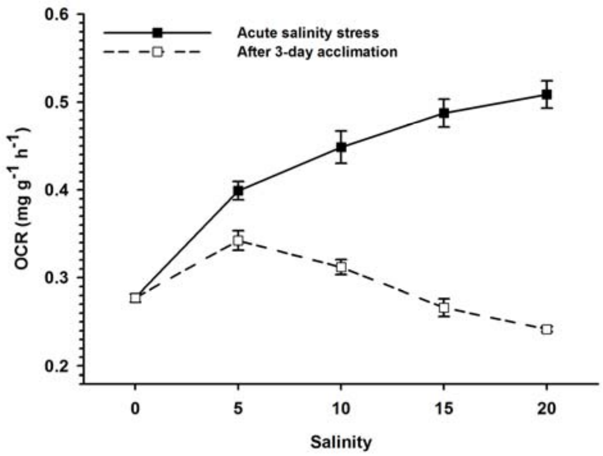

Fig. 1. Oxygen consumption rate (*OCR*) of juvenile Chinese sturgeon *Acipenser sinensis* under acute salinity stress and 3-days after acclimation to different salinities

Table 3. Mean body weight (*BW*), oxygen consumption rate (*OCR*) and sample size (N) of the juvenile Chinese sturgeon *Acipenser sinensis* at different Illumination exposures

Illumination (lx)	*BW* (g)	N (ind.)	*OCR* (mg/g/h)*
0	5.58	9	0.225 ± 0.013[a]
500	5.28	9	0.341 ± 0.016[b]
2000	5.99	9	0.405 ± 0.004[c]
20000	5.78	9	0.410 ± 0.013[c]

*Means with different letters in the same columns were significantly different ($P < 0.05$).

Effects of Cu^{2+} on OCR

The *OCR*s of the control group and 4 Cu^{2+} concentration groups were measured when the water temperature was 15 ºC with body weight averaging 14.87 ± 0.42 g. There were significant differences between the control group and Cu^{2+} concentration groups ($P < 0.01$, Fig. 2) with an obviously negative correlation between *OCR* and Cu^{2+} concentration (*C*): $OCR = 1.748 - 0.009\ C$ ($R^2 = 0.964$, $P < 0.01$). The highest value of *OCR* in control group was 0.265 mg/g/h. When the Cu^{2+} concentration reached to 8 mg/l, the *OCR* dropped to the lowest value, decreased by 54.3 % compared with the control group. The relationship between Cu^{2+} and the *OCR* could be described as $OCR = 0.237^{e-0.09\ C}$ ($R^2 = 0.898$, $P < 0.01$).

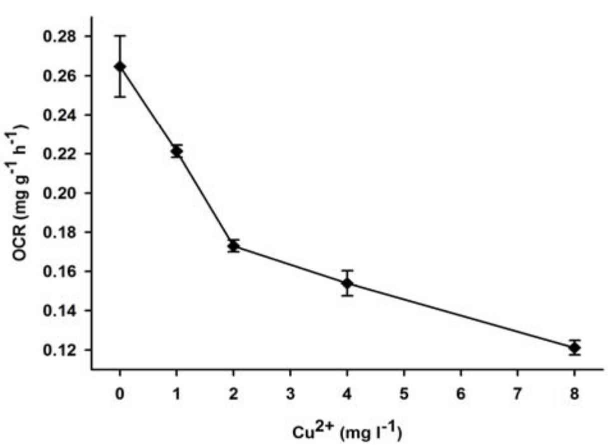

Fig. 2. Oxygen consumption rate (*OCR*) of juvenile Chinese sturgeon *Acipenser sinensis* at different Cu^{2+} concentrations

Discussion

The standard metabolism of fish refers to the basic metabolism with being inactive, fasting and undisturbed. Jobling et al. (1985) suggested that the standard metabolism is comprised of components: the energy of organization repair and renovation; the second one is the energy of maintaining the inner environment stable.

The *OCR*, tested in the present study, is only an approximation of the standard metabolism, since fish showed some swimming activity in the test chambers (Chen and Xie 2002). The *OCR* is a very important indicator, which can reflect the physiological state, and signal stress conditions influenced by external factors.

Effects of temperature on *OCR*

Temperature is one of the most important factors that influence the respiratory metabolism and determine the activity level of all biochemical processes in organs and tissues, in particular enzyme activities. Some researchers founded that it could lead to physiological malfunction, particularly the *OCR* would decrease rapidly when the fish like striped beakfish *Oplsgnathus fasciatus* (Yan et al. 2007) and black seabream *Acanthopagrus schlegeli* (Wang et al. 2007) were not living in the suitable temperature range. In the present study, it was shown that the OCR of the 2 months old juvenile Chinese sturgeons increased with increasing of water temperature when the water temperature ranged from 5 ºC to 30 ºC, but altered when the water temperature was out of that range, indicating that juvenile Chinese sturgeon may adapt to the water temperature to some extend.

Q_{10} is used to judge the effects that the water temperature change acts on the fish metabolism rate, when the temperature changes in a small scale. In this study, the *OCR* undoubtedly increased, but the increasing scale gradually decreased when the water temperature increased every 10 ºC. In fact, it indicated that the effects of water temperature on metabolism decreased with the increase in temperature ranging from 5 to 30 ºC, which was consistent with the study done on juvenile catfish *Pelteobagrus fulvidraco* (Liao et al. 2004).

The Q_{10} of juvenile Chinese sturgeon reached to the 2.2291, the highest Q_{10} when the water temperature increased from 5 to 15 ºC. Probably, the basic metabolism of juveniles was unstable during this water temperate range. It has been reported that the juvenile Chinese sturgeon could not survive when the water temperature was below 15 ºC (Yang, et al. 2008). However, when the water temperature increased from 20 to 30 ºC, the Q_{10} was 1.4251, which reaches to the lowest value. The second lowest Q_{10} appeared when the water temperature varied from 15 to 25 ºC, indicating that from 15 to 30 ºC, the basic metabolism of juveniles little changed. The physiological metabolism was very stable in this temperature range, indicating a fit temperature range for early breeding of the juveniles. Similarly, the most suitable temperature range for raising juvenile Chinese sturgeon was 18-22 ºC in previous studies (Zhu et al. 2006; Yang et al. 2008). The present results may provide theoretical evidence of the most suitable temperature for raising and protecting juvenile Chinese sturgeon.

Relationship between suffocation point and dissolved oxygen

The suffocation point of fish can directly reflect the ability of endure the low oxygen environment, which is mainly determined by the species, the age of fish, and the environment factors such as living conditions, the feeding habit, etc. In the present study, the suffocation points (1.445 - 1.725 mg/l) of juvenile Chinese sturgeon were higher than those of other fish species. For instance, the suffocation point is 0.32 mg/l in red common carp *Cyprinus carpio* (body weight, 31.8 g; water temperature, 23 ºC) (Hu et al. 1991), 0.15 mg/l in mirror carp *C. carpio* (body weight, 30 - 70 g; water temperature, 20ºC) (Fan and Liu 1991), 1.073 mg/l in chinese sucker (body weight, 6.75 g; water temperature, 20 ºC) (Pan et al. 2007), 1.36 - 1.32 mg/l in amur sturgeon (body weight, 10.2 - 30.2 g; water temperature, 20 ºC) (Song et al. 1997), 1.55 mg/l in red sea bream *Pagrosomus major* (Dong and Zhang 1992), 1.6 mg/l in sevruga *A. stellatus* (Dettlaff and Ginzburg 1958), and 2.81 mg/l in paddlefish (body weight, 156.4 g; water temperature, 19 - 20 ºC) (Xu et al. 2008). These studies supported that the juvenile Chinese sturgeon consumed high oxygen, which could bear high suffocation point. In the larval rearing phase, the stocking density and the dissolved oxygen should be paid much more attention. The water should be changed frequently when the temperature is high, in case the fish lacks oxygen.

Effects of salinity on OCR

Salinity is also one of the most important factors that affect the inner environment stability. As to some fishes which can live in a large scale of salinity, with the salinity altered gradually, the inner environment which is disturbed will adapt to the change (Iwama et al. 1997). The Chinese sturgeon can live in a large scale of salinity and it is also a kind of migratory fish (Zhuang et al. 2002). It starts to migrate from the youth, in the middle and last ten-day of May every year. The youth migrates to the estuary of Yangtze River, and it will stay in the estuary up to the last ten-day of August.

During this period, it must fulfill the osmoregulation, preparing to migrate to the sea (Yi 1994; Zhuang et al. 2006). In the present study, 3 days *OCR* of juvenile Chinese sturgeon through acute salinity stress was studied. It showed that the *OCR*s increased with increasing of salinity, and the fish could allocate some energy to sustain the balance of salinity through respiratory metabolism. The study also indicated that the *OCR*s altered dramatically from fresh water to the salinity of 5, indicating the respiratory metabolism of juveniles alters sharply under this condition. However, when the salinity ranged from 5 to 20, the respiratory metabolism of juveniles altered smoothly, indicating that the respiratory metabolism changed mildly in this salinity range in juvenile Chinese sturgeon. The *OCR* altered dramatically after acclimation to the different salinity groups for 3 days. The *OCR*s increased firstly and then decreased with increasing of salinity, the *OCR* reached to the acme when the salinity was 5 finally. Compared with the controls, the *OCR* was still higher when the salinity was 10, which changed when the salinity was 15 and 20.

Thus, it was evident that Chinese sturgeon can regulate the osmotic pressure at its early life stage. The juveniles needed higher respiratory metabolism energy to regulate the osmotic pressure and kept it balanced when they lived under a relative lower salinity environment. While lived in a higher salinity environment such as at the salinity of 15 and 20, the environmental osmotic pressure was higher than the body osmotic pressure, which might lead to a disturbed normal metabolism, a decreased oxygen consumption rate, and death at the end. In addition, after 3 days acclimation to the different salinity groups, the *OCR*s lightly decreased in all of the experiment groups compared with the initial rates. The decrease range increased with the increase of the salinity.

Effects of illumination on OCR

According to the different *OCR*s, the metabolism level can be divided into 3 kinds: (I) the *OCR* was higher on daytime than at night. In the present study, The fish individuals needed higher dissolved oxygen to keep active at daytime when it stopped moving at night, which is supported by some studies on other species such as silver sea bream *Rhabdosargus sarba*, striped beakperch *Oplegnathus fasciatus*, requiem shark *Carcharhinus limbatus*, reeves shad *Macrura reevesii*, and herring *Clupea harengus*. (II) The *OCR* was higher at night. The juvenile Chinese sturgeons kept motionless on daytime while they were very active at night. Similar results have been found in greenling *Hexagrammos otakii* and Armoured cusk *Hoplobrotula armata*. (III) There were no differences of the *OCR*s between daytime and night in Chinese Stingray *Dasyatis sinensis*, Yellowcheek *Elopichthys bambusa*, amur sturgeon and white spot pike *Esox lucius* (Qiao et al. 2005). The present results showed that the *OCR*s were significantly different between the illumination groups and dark groups with a higher amount in illumination groups. It indicated that the juvenile Chinese sturgeon belongs to the first kind metabolism, but further study is needed to prove this inference.

Effects of Cu^{2+} concentration on OCR

In the present study, with increase of Cu^{2+} concentration, the *OCR*s of juvenile Chinese sturgeon decreased gradually. Compared with the controls, the *OCR* declined by 54.3%, and it indicated that Cu^{2+} was toxic to the juvenile Chinese sturgeon. Gills can accumulate much more Cu^{2+} than other organs when the juveniles were raised in different Cu^{2+} concentration, and thus the respiratory metabolism became the first victim of Cu^{2+}. Similarly, it had been reported that Cu^{2+} could inhibit the activity of Ca^{2+}-ATP enzyme, and could also enhance the number of chloride cells, make the ultra-structure changed and the osmotic mechanism disturbed (Chai et al. 1990; Huang et al. 1994). Furthermore, the gill and skin mucus increased when the Cu^{2+} existed.

The mucus was a double edged sword which could not only prevent the macromolecular substances from injuring the gill organization but also could bind with the metal protein substances to prevent the gas exchange of gill epithelium (Jia 2001). The *OCR*s of juvenile loach *Misgurnus anguillicaudatus* decreased dramatically with the extension of exposure time under exposed to the 6 mg/l Cu^{2+} in one hour at 26 ºC (Jia 2001). As a result, the initial *OCR* lightly enhanced after exposed to the Cu^{2+}, however, after 40 minutes acclimation to the living condition, the *OCR* dropped by 94.2% relative to the controls, indicating that the Cu^{2+} concentration may play a key role in affecting the respiratory metabolism and it may be a very toxic substance to the Chinese sturgeon at the early life stage.

Conclusions

Temperature, salinity, illumination and Cu^{2+} affect the *OCR* as well as the metabolic requirements of the Chinese sturgeon in their juvenile stages, which would have a significant influence on the successful hatching of fertilized eggs and larval development as well as the ongrowing of other life cycle stages of the Chinese sturgeon. Thus, the present results provide us with some useful guidelines which may be applied into protection and cultivation management of this endangered species.

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