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The effect of some water parameters on the oxygen consumption rate of embryos and larvae of the Chinese Sturgeon (*Acipenser sinensis*)

By LIU Jian-Yi[1,2] , WEI Qi-Wei[1,2], YANG De-Guo[1,2], TANG Guo-Pan[3], DU Hao[3], ZHU Yong-Jiu[1,2]

[1]*Key Lab of Freshwater Fish Germplasm Resources and BiotechenlogyMinistry of AgricultureYangtze River Fisheries Research InstituteChinese Academy of Fishery SciencesJingzhou* 434000; [2]*Freshwater Fisheries Research CenterChinese Academy of Fishery ScienceWuxi* 214081: [3]*Fishery College, Huazhong Agricultural UniversityWuhan 430070, China*

Summary

The effect of pH, salinity, total ammonia-N and alkalinity on oxygen consumption rate (OCR) were studied in embryos and larvae of the Chinese Sturgeon *(Acipenser sinensis)* with closed volumetric flasks. The OCR of embryos increased with pH values rising from 5.5 to 7.0, but decreased with higher pH values rising from 7.0 to 9.0.The optimum pH as a single variable was determined to be between 6.5 and 7.5.OCR of embryos and larvae increased as the water salinity increased from 1.25to 20, but it became stable when salinity was higher than 40In water containing 1.07 mg/L to 34.15 mg/L of total ammonia-N both respiration frequency and OCR increased with the increase of concentration of total ammonia-N, but decreased when concentration was higher than 34.15 mg/L.OCR of larvae increased with alkalinity.

Introduction

The Chinese Sturgeon (*Acipenser sinensis*) is mainly distributed along southern latitudes of China. It is a species of highly commercial but also academic significance in relation to phylogeny and zoogeography of fishWang et al, 2002. Overfishing, hydro-dam construction and environmental pollution have greatly affected its natural ecological system and the population is seriously endangered, despite its listing by Chinese government as a "First-Class Animal for Protection" in 1988. Since about two decades, artificial propagation for restocking its natural population has been conducted. According to the data collected in the estuary of Yangtze River in 1999 and 2002, the artificially released Chinese sturgeon only accounted for less than 3% of the captured sturgeon, which demonstrated that, although restocking of the Chinese Sturgeon has some positive effects on the population, its natural propagation still plays the dominant role in recruitment (Wei , 2003) .

The oxygen consumption rate (OCR) of fish is an important factor in metabolism. The amount of dissolved oxygen plays a key role in the successful hatching of fertilized eggs and larval development. A study of OCR of the young Chinese Sturgeon under different environmental conditions will help to understand the metabolic demands, which are of great significance in the ecology of the species, and also for cultivation. Substantial work has been conducted globally on OCR of fish embryos and larvae for both environmental and aquaculture research. Intensive studies have focused on the effect of water pollution on the physiology of fish such as respiration, cardiac rhythm and metabolism and food conversion . Results showed that the opercular movement of fish can be employed as a basic method to study the effect of several types of water pollution that affect fish respiration (Hochleithner and Gessnery,1999; Huang 1990). In China, the only study on OCR of the Chinese Sturgeon larvae was carried out by Xie et al (1989). There is no report available on the OCR of the embryos and larvae of the Chinese Sturgeon under different environmental conditions, in particular some key physicochemical parameters such as pH value and ammonia concentration, which are important in intensive cultivation. In this study, the authors investigated the differences in OCRs for early Chinese Sturgeon embryosbody encircling the yolk: so-called „tail-close-to-heart stage"and early larvae under different pH, salinity, total ammonia-N and $NaHCO_3$ concentrations as single variable factors in order to gain some understanding about the metabolic response to water quality changes in fertilized eggs and larvae. Different developmental stages of the Chinese Sturgeon were identified according to the standard description used by Yin 1995.

Material and Methods

Test organisms

The embryos and larvae were obtained from the artificial propagation programme for the Chinese Sturgeon at the Yangtze River Fisheries Institute in October 2003. The water source used in this study was that from the Propagation and Restocking Experimental Base for Chinese Sturgeon in Fenghuangshan, Jingzhou City, Hubei Province.

Methods

The experiments were carried out using the method described by Liu et al. (1995). The device was the same used for OCR determinations with the Chinese Giant Salamander embryos. The respiration chamber consisted of a gas-proof flask with a volume of 285 ml. Dissolved oxygen in the water was determined by the Winkler method. The study was performed in two experimental groups with one of them as the control (without larvae or embryos inside). Embryos and larvae in each experiment were from the same batch. Before the start of the assay, larvae and embryos were delivered to the experimental water 1-2 hours earlier in order to allow some acclimation to the test environment. After that time, they were filled in the test flasks and sealed. Time, temperature and fish batch were recorded. Water used for the preparation of the experimental

test waters was ion-free pure water. Dissolved oxygen in the water was determined to guarantee to be initially always higher than 8.0 mg/L. An YSI-581 oxygen meter (USA) was used for this purpose. Analytical-grade chemicals were used to prepare the various test concentrations. Water temperature was maintained stable by placing the test flasks in a water bathpH and ammonia = 20.2 ± 02°C; salinity=20.5 ± 02°C alkalinity =21 ± 02°C. During the tests, respiration flasks were inverted for 10 times every 15 minutes runtime to ensure good mixing and even distribution of dissolved oxygen. Test flasks were placed in the dark and direct light exposure was prevented. After embryos and larvae respired in the flasks for a period of time (60 minutes for embryos and 90 minutes for larvae), water of the flasks was siphoned out and oxygen concentration determined. Data were collected from two replicates and the average value was used. Water temperature was measured with a calibrated mercury thermometer and the pH was determined with pH-sensitive indicator paper. OCR was calculated as follows:

Oxygen consumption (mg/ind/hr) = A_1-A_2/N/T

Where A1 and A2 represent the amount of dissolved oxygen in the control and test flasks (mg) respectively, N = number of individuals in the flasks, and T=respiration time (hr). From these data the consumption per individual and unit time has been calculated.

Results

The effect of pH on the OCR of embryos

Results on OCR at the "rolling"-embryos stage are shown in Table 1. The test pH values ranged from 5.5 to 9.0. While the OCR were very high at low pH, consumption values decreased from pH 5.0 to a minimum at about pH 7.0 but increased at higher pH values again to similar levels as in very low pH tests, indicating that at values around the neutral point there seems to be the lowest stress for pH as determined by oxygen consumption which reflects metabolic rate.

The effect of salinity on OCR

Because Chinese Sturgeon spawns in freshwater and grows to maturation in the sea, it can be assumed to osmoregulate very well. However, it is uncertain whether this also holds true at early life cycle stages. Therefore, we conducted tests on the effect of salinity on the OCR of the embryos and larvae. Six salinity concentrations were produced reflecting 1.25, 2.5, 5, 10, 20, 4 of water source in the Base. pH in the tested water was 6.4. OCR results for the embryos tail-close-to-heart stageand larvae (average weight 82.1 ± 1.7mg, n = 29) are presented in Figure 1. It demonstrated that, when low salinity was presented, OCR for the embryos increased slightly while that of larvae increased significantly. When salinity was 20 or higher, the OCR of embryos and larvae was relatively stable. We also observed that, when salinity of the water was higher than 40, the embryos membrane was crumpled. The larvae were uneasy with higher respiration rate and subsequently quivered on the floor, which would die within approximately 7 minutes. In 20 salinity, all the phenomena abovementioned occurred to the larvae, but two of the larvae survived with their tails quivering on the floor when the experiment was completed.

The effect of total ammonia-N on the OCR

Seven total ammonia-N concentrations were prepared1.07, 2.13, 4.27, 8.54, 17.07, 34.15 and 68.29 mg/L for the tests, the pH of the water was 6.8 and the water temperature was 20.0 ± 0.2°C. The

Table 1
Effects of pH on OCR of embryosrolling-embryos stageof *Acipenser sinensis*

pH	Number of embryos	Replicates	OCR (ig/ind/hr)
5.5	50	2	5.60
6.5	50	2	4.00
7.0	50	2	2.20
7.5	50	2	2.40
8.0	50	2	3.68
8.5	50	2	4.00
9.0	50	2	4.40

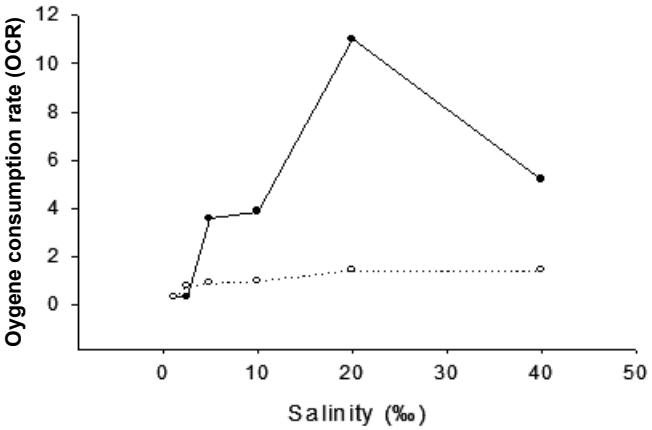

Fig.1. Effects of salinity on the OCR of embryos (tail-close-to-heart) and larvae (average weight 82.1 ± 1.7mg, n = 29) of *Acipenser sinensis;* Embryos (µg/ind/hr) = dots; Larvae (mg/ind/hr) = open circles

OCR of embryosrolling-embryo stageand larvae average body weight 30.2 ± 1.3 mg, n = 20 gradually increased with the total ammonia-N concentration 1.0768.29 mg/L) to a certain point and then decreased, as shown in Figure 2.

The effect of alkalinity on the OCR

Alkalinity test solutions were produced by adding different amount of $NaHCO_3$ to the water to produce the $NaHCO_3$ concentrations to be 0, 10, 20, 40, 60, 80 and 100 mg/L. The pH values in this experiment were at all alkalinity levels within a narrow range near optimum, ranging between 6.85 and 7.35. The respective OCRs obtained for of the larvae (average body weight 54.7 ± 1.6 mg, n = 39) exposed to different alkalinities are shown in Table 2. With increasing alkalinity in the water, OCR values for larvae of the given size also increased. While the OCRs were only slightly elevated at alkalinity values up to 20 mg/L, there was a drastic increase at higher alkalinities, leading to eight-fold increase at 100 mg/L. At higher alkalinities larvae exhibited clear signs of uneasiness in their behavior but also should massive mucus production at their gill.

Discussion

The effect of pH on the OCR of embryos

The experiments clearly showed that pH has an effect on metabolic rates of the embryos. We also observed that, hatching time of the

Table 2
Effects of alkalinity on the OCR of larvae (average body weight 54.7±1.6 mg) of *Acipenser sinensis*

$NaHCO_3$ (mg/L)	pH	number of larvae per test	replicates	OCR (mg/ind/hr)
0	6.85	20	2	0.594
10	6.90	20	2	0.603
20	6.95	20	2	0.594
40	7.10	20	2	0.871
60	7.25	20	2	1.073
80	7.32	20	2	1.109
100	7.35	20	2	4.753

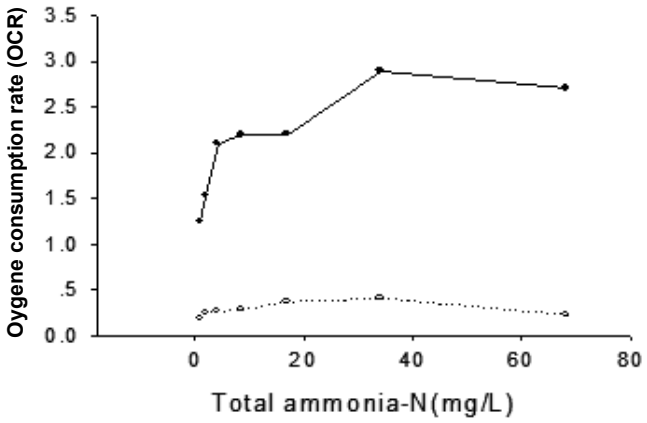

Fig.2 Effects of total ammonia-N on OCR of embryosrolling-embryos stageand larvaeaverage body weight 30.2 ± 1.3 mg, n = 20of *Acipenser sinensis;* Embryos (μg/ind/hr) = dots; resting larvae (mg/ind/hr) = open circles

eggs advanced at low pH and high pH values, an observation which is in accordance with results published by Zhang and Li (1992), , indicating that with fertilization and hardening of the egg-shell, the eggs absorb water and swell up at different levels while the egg membrane thins down. In the presence of high acidity or high alkalinity, embryos will lose their ion balance and eventually die. Even though hatched, the larvae will be dark, thin and extremely weak (unable to swim freely). Although fertilized eggs can resist acidity to a certain extent, when the pH is low, the activity of the hatching enzyme will be activated early resulting in an advanced softening of the egg membrane and premature hatching. The resultant larvae will be weak and show high mortality rates. In the early yolk sac larvae stage, acidic water will decrease the transformation efficiency of the yolk nutrients (Rarker and Mckeown, 1989). However, high pH will affect their respiration as well, causing the larval dermis and gill tissue to deteriorate with subsequent effects on survival. The results of our study suggest that pH values between 6.5 to 7.5 are appropriate for hatching and rearing of the Chinese sturgeon larvae.

The effect of salinity on the OCR of embryos and larvae

Salinity of water could significantly influence the OCR of embryos and larvae of the Chinese sturgeon. OCR levels increased with the increase in salinity which can be explained with the energy requirements to modulate osmosis at higher salinities. In our experiment, the OCR increased more rapidly with increasing salinity for larvae than for embryos which can be explained with the differences in metabolic rates. Salinity effects, however, are less pronounced than pH effects although very low salinities close to freshwater should be the preferred medium when considering the mass cultivation. . .

The effect of total ammonia-N on the OCR

Ammonia may be present as two different chemical species, NH_3 (unionized) and NH_4^+ (ionized), whose equilibrium is affected both by water temperature and by pH (Pan et al, 1993). In fish, NH_3 is the more toxic component of the two forms. In our study, pH is 6.8 and temperature is 20.20.2. when total ammonia-N increased from 1.07 mg/L to 34.15 mg/L, the OCR of the embryos and larvae increased gradually. But when total ammonia-N increased from 34.15 mg/L to 68.29 mg/L, the OCR of larvae and embryos decreased gradually. This may demonstrated that, in the early stage, total ammonia-N could stimulate the metabolism that resulted in higher respiration rate; in the late stage, high ammonia-N concentration led to the toxicosis state of the larvae and embryos, in which the larvae was inert with low respiration rate and thus, low OCR. Therefore, in the process of the cultivation of the embryos and larvae of the Chinese Sturgeon cultivation, ammonia-N should be controlled in a proper range so that the embryos and larvae could develop well.

The effect of alkalinity on the OCR on larvae

HCO_3^- is a weak acidic ion. It can bind to H^+ to consume acid in the water, which is the reason of alkalinity of the water. Therefore, it interacts with salinity (Zhang and Li, 1992). Alkalinity can erode the fish tissue, especially the gill and it can affect the respiration and air exchange of fish and Boer Phenomena occurs. This could restrain fish growth and even result in mortality (Lei et al, 1985). Meanwhile, HCO_3^-could bind with H^+ to produce CO_2 in the water. The increase of dissolved CO_2 in water is also toxic to fish. The toxicity mechanism is that, in water of high CO_2 concentration, CO_2 in the fish blood could not diffuse into the environment, which results in the increase of CO_2 or H_2CO_3concentration and low pH in fish blood. This will affect the combination ability of Hb to O_2, which results in O_2 shortage in the blood and eventually death.

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Author's address: Corresponding author Wei Qiwei
NO.41 Jianghan North Road,
Jingzhou, Hubei 434000
P.R.China.
E-mail: weiqw@yfi.ac.cn
First author: E-mail: LIUjy999@vip.163.com