Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Effects of total dissolved gas supersaturated water on lethality and catalase activity of Chinese sucker (*Myxocyprinus asiaticus* Bleeker)*

Shi-chao CHEN[1], Xiao-qing LIU[2], Wen JIANG[1], Ke-feng LI[†‡1], Jun DU[†‡3], Dan-zhou SHEN[3], Quan GONG[3]

(*[1]State Key Laboratory of Hydraulics and Mountain River Engineering, Sichuan University, Chengdu 610065, China*)
(*[2]School of Energy and Environment, Xihua University, Chengdu 610039, China*)
(*[3]Fisheries Institute, Sichuan Academy of Agricultural Sciences, Chengdu 611731, China*)
[†]E-mail: kefengli@scu.edu.cn; dujun9100@yahoo.com.cn

Received Jan. 16, 2012; Revision accepted May 30, 2012; Crosschecked Sept. 10, 2012

Abstract: Total dissolved gas (TDG) supersaturation caused by dam sluicing can result in gas bubble trauma (GBT) in fish and threaten their survival. In the present study, Chinese suckers (*Myxocyprinus asiaticus* Bleeker) were exposed to TDG supersaturated water at levels ranging from 120% to 145% for 48 h. The median lethal concentration (LC_{50}) and the median lethal time (LT_{50}) were determined to evaluate acute lethal effects on Chinese suckers. The results showed that the LC_{50} values of 4, 6, 8, and 10 h were 142%, 137%, 135%, and 130%, respectively. The LT_{50} values were 3.2, 4.7, 7.8, 9.2, and 43.4 h, respectively, when TDG supersaturated levels were 145%, 140%, 135%, 130%, and 125%. Furthermore, the biological responses in Chinese suckers were studied by assaying the catalase (CAT) activities in gills and muscles at the supersaturation level of 140% within LT_{50}. The CAT activities in the gills and muscle tissues exhibited a regularity of a decrease after an increase. CAT activities in the muscles were increased significantly at $3/5LT_{50}$ (P<0.05) and then came back to the normal level. However, there were no significant differences between the treatment group (TDG level of 140%) and the control group (TDG level of 100%) on CAT activities in the gills before $3/5LT_{50}$ (P>0.05), but the activities were significantly lower than the normal level at $4/5LT_{50}$ and LT_{50} (P<0.05).

Key words: Total dissolved gas supersaturation, Median lethal time (LT_{50}), Median lethal concentration (LC_{50}), Chinese sucker, Catalase
doi:10.1631/jzus.B1200022 **Document code:** A **CLC number:** Q17

1 Introduction

The flood sluicing from a dam will carry atmospheric air and dissolve it when hitting the plunge-pool, and can become supersaturated with gas under high hydrostatic pressure (Gale *et al*., 2004). Since the 20th century, total dissolved gas (TDG) supersaturated water has been considered a threat to aquatic resources, and is considered a water quality issue. Many studies have been carried out to explore the effects of TDG supersaturation on fish. Huang *et al*. (2010) found that juvenile rock carp could survive at supersaturated levels below 120% and showed a strong avoidance response to the supersaturated water with gas saturation above 135%. Salmon and rainbow trout could always avoid 145% and 125% saturation, but were dull to 115% saturation (Stevens *et al*., 1980). Gray *et al*. (1985) reported that the 96 h LC_{50} values of sea bass and striped mullet were 127.2% and 129.4% respectively at 20 °C. No significant changes of growth rate were found by Liu *et al.* (2011) after rock carp were exposed to gas saturation ranging from 100% to 116% for 42 d, but biochemical responses assessed by catalase (CAT) and superoxide dismutase (SOD) activities were obviously found.

‡ Corresponding authors
* Project supported by the National Natural Science Foundation of China (No. 50979063) and the Scientific Research Foundation for Young Teachers of Xihua University (No. Z1120412), China

As existing findings are applicable to specific species or rivers only, research on TDG supersaturation thus far has not been sufficient to set up a limit level for water quality assessment in China. With high or superhigh dams being built upstream of the Yangtze River in recent years, the issue of TDG supersaturation is becoming more and more serious. TDG levels above 138% were observed downstream from the Three Gorges dam and the Ertan dam by field observation during dam sluicing (Li *et al*., 2009; Qu *et al*., 2011). Fish were found dead from gas bubble disease downstream from the Three Gorges dam (Tan, 2006). Chinese suckers dwelling in the Yangtze River were threatened by TDG supersaturation. However, existing research mainly focuses on the current resource situation, reproduction and artificial culture, molecular biology and fodder (Chen, 2008). The effect of TDG supersaturation on Chinese suckers has not been reported so far. In this study, the median lethal concentration (LC_{50}), the median lethal time (LT_{50}), and the CAT activity of tissues in the Chinese sucker were measured to evaluate the effects of acute lethality and the biological response in fish exposed to TDG supersaturated water. It can be expected that the research results will provide scientific data for operational schemes of hydropower plants and the formulation of water quality standards in China.

2 Materials and methods

2.1 Experimental fish and instrument

The present study was carried out by using half-year-old Chinese suckers (*Myxocyprinus asiaticus* Bleeker) which were transported from Sichuan Fisheries Research Institute, China, in November 2011. Before the experiment, they were placed in tanks of the State Key Laboratory of Hydraulics and Mountain River Engineering (Sichuan University, China) for 8 d to adapt to living conditions with stable water temperature at 21 °C and pH 7.1–7.8. The fish were fed on *Limnodrilus hoffmeisteri* every day, but were not fed during the trial. Water temperature, TDG level, pH value, and dissolved oxygen (DO) value were monitored daily using a mercury thermometer, point four tracker (Point Four Systems Inc., Canada), digital pH meter (JENCO Model 6010, China), and dissolved oxygen meters (Oxi 3210 SET 3 Inc., Germany), respectively. The experimental conditions were as follows: water temperature 21 °C, pH 7.1–7.8, and DO 7.5–8.0 mg/L. The weight of the fish was (39.54±7.63) g (mean±standard deviation (SD)), while the length was (11.54±0.91) cm (mean±SD).

2.2 Acute lethality experiment

The experiment system was based on Huang *et al*. (2010) to generate the TDG supersaturated water. Twenty fish were held in each tank with TDG levels of 145%, 140%, 135%, 130%, 125%, 120%, and 100% for 48 h, respectively. The behaviour of fish was observed, and the death time of each fish was recorded during the trial. The LT_{50} was calculated in probit methods, while the LC_{50} was analyzed through linear interpolation (Zhou and Zhang, 1989).

2.3 Measurement of CAT activity

The group with the supersaturated level of 140% was selected to conduct the biological response experiment according to the LT_{50} calculated in Section 2.2. Forty fish were used in the treatment group (TDG level of 140%) and the control group (TDG level of 100%), respectively. The repeated test was carried out at the same time. Three fish from both the treatment group and control group were sampled every other $1/5LT_{50}$. They were narcotized with MS-222 immediately and weight and length were measured. Gills and muscles with a weight of 1.5 g were excised from each fish and ground under liquid nitrogen, and then they were extracted with the method described by Wang *et al*. (2010). The 1.5 ml reaction mixture liquid contained 0.05 mol/L sodium phosphate buffer (pH 7.0) and 0.1 mmol/L ethylene diamine tetraacetic acid (EDTA). The enzyme extraction was gained by centrifugation at 4 °C. The CAT activity was determined by assaying the supernatant with an ultraviolet spectrophotometer according to Montavon *et al*. (2007). The enzyme-activity unit of CAT was defined as the amount of the enzyme which caused a decrease of 0.01 on the absorbance of 0.6% (v/v) hydrogen peroxide (H_2O_2) per min under the wave length of 240 nm.

2.4 Statistical analysis

The software SPSS was used to analyze the differences of CAT activities between the treatment group and the control group. The level of significant differences was set at $P<0.05$. The figures of LC_{50}, LT_{50}, and the changes of the CAT activities were determined using Excel 2003.

3 Results

3.1 Acute lethality

At the beginning of the trial, fish in each group swam everywhere in the tanks. After a few minutes, they gathered together at the corner of the tanks. Fish in the tank with the TDG level of 145% began to swim alone after 1 h. Some fish started to swim quickly and suddenly, but were slow in reacting about 1 h later. The phenomenon of sidestroke was observed. Sometimes they leaped in the air and then fell at the bottom of the tank without swimming ability. After a while, these phenomena repeated constantly until they died on the water surface with mouths open and lots of bubbles in the fins. Fish in the groups of 140%, 135%, 130%, and 125% showed the same phenomena as those in the group of 145%. On the other hand, no dead fish were found in the group of 120% or 100%.

The LT_{50} and LC_{50} are shown in Figs. 1 and 2. The Chinese suckers died a few hours after the trial started with levels greater than 125%. However, the LT_{50} dramatically increased when the gas saturation level was 125%. The LT_{50} was not measured in the group with the gas saturation level of 120%, for no dead fish were found in it. Since all fish in the groups of levels above 125% died within a few hours, the LC_{50} was calculated at 4, 6, 8, and 10 h, respectively.

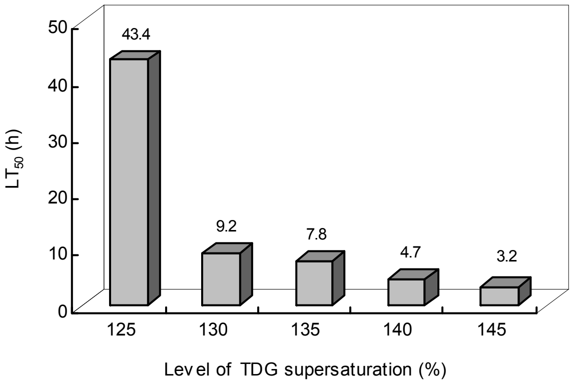

Fig. 1 LT_{50} for Chinese suckers at different TDG supersaturations

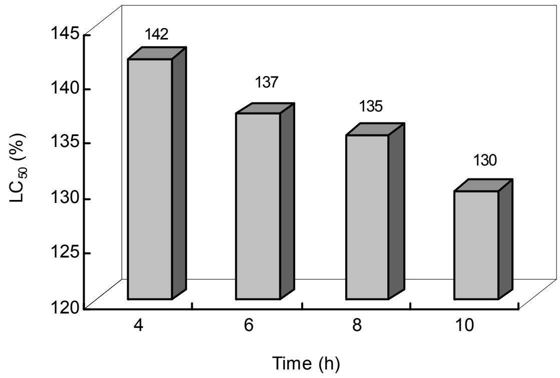

Fig. 2 LC_{50} for Chinese suckers at different time points

3.2 CAT activity

As shown in Fig. 3, CAT activities varied with different TDG levels, exposure time, and tissues. As shown in Fig. 3a, the CAT activities in muscles of Chinese suckers exposed to gas saturation level of 140% first increased and then dropped. They were significantly ($P<0.05$) increased to the maximum at $3/5LT_{50}$ in the treatment group compared with the control group. And then they dropped back to the normal level ($P>0.05$).

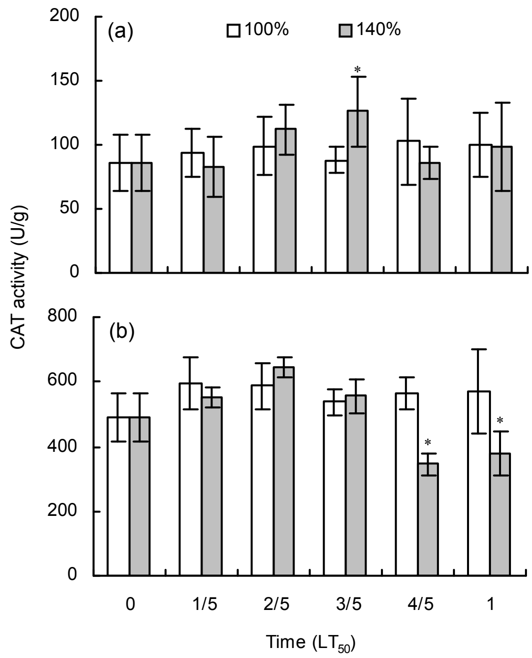

Fig. 3 Changes of CAT activities in the muscles (a) and gills (b) of the treatment and control groups

Values were expressed as mean±SD. * $P<0.05$

Fig. 3b showed the same tendency in gills as that in Fig. 3a, but no significant differences were found between the treatment group and the control group during the increasing stage (P>0.05). They began to drop after $3/5LT_{50}$, and were significantly lower in the treatment group than in the control group at $4/5LT_{50}$ and LT_{50} (P<0.05).

4 Discussion

4.1 Influence factors on the lethality by TDG supersaturation

Many studies have shown that different tolerances for TDG supersaturation occurred in different fish. Beeman *et al*. (2003) described the LT_{50} of five different fish in the Columbia River. Table 1 shows that the sensibility to TDG supersaturation varies in different fish under the same condition. Compared with the fish in Table 1, Chinese sucker showed a moderate-level tolerance to TDG supersaturation.

Table 1 LT_{50} for five different fish at 125% and 130% TDG supersaturations*

Species	LT_{50} (h)	
	125%	130%
Largescale sucker	17.0	9.5
Longnose sucker	56.0	30.0
Northern pikeminnow	15.3	10.5
Redside shiner	116.0	31.0
Walleye	169.0	62.0

* Data are from Beeman *et al*. (2003)

Results indicated that the tolerance to TDG supersaturation varied with age. Arntzen *et al*. (2009) found that the chum salmon egg had a strong tolerance to the supersaturation levels ranging from 110% to 115%. Bohl (1997) pointed out that steelhead trout fry could survive when the supersaturation level was 102%, but serious traumas were found in gills, skeletons, vessels, and the nervous system. However, the initial level causing the death of adult salmons was 116%.

Some factors played an important role in lethality of fish exposed to TDG supersaturation, such as water temperature and supersaturation levels. Nebeker *et al*. (1978) found that mortality of the rainbow trout and the Chinook salmon increased with rising temperatures. However, the silver salmon and the red salmon were not affected. Alderdice and Jensen (1985) and Jensen *et al*. (1986) reported that fish in the groups with high TDG levels died earlier than those with lower TDG levels. Huang *et al*. (2010) indicated mortality increased with the rising TDG level. Thus, keeping the experimental conditions steady was of great significance, such as stable temperature at 21 °C.

Furthermore, there are different tolerances in different fish exposed to the TDG supersaturated water owing to the individual differences of fish. In this study, the fact that three fish were sampled from each group every time and the repeated test was carried out at the same time could decrease individual differences.

This study showed the similar regularity as that in Huang *et al*. (2010). The LT_{50} in the group of 130% dramatically increased compared with the group of 125%. Moreover, no dead fish were found in the group of 120%. Under the experimental conditions, the threshold of the acute TDG level in Chinese sucker could be determined at 125%. However, TDG supersaturation of 138% has been measured downstream from the Three Gorges dam with height of 185 m. And at the upper reaches of the Chinese sucker protection zone, dams more than 200 m high, like Xiluodu dam and Baihetan dam, are being built or prepared. TDG levels in the protection zone may be getting much higher than 125%. Not only the survival is under threat, but also the reproduction may be seriously affected, which needs further studying.

4.2 CAT activity

As is known, the reactive oxygen species (ROS) including oxygen radical and H_2O_2 are being produced during the oxygen metabolism. H_2O_2 is being transformed to hydroxyl radicals easily, which does harm to cells. However, it can be cleared away by CAT efficiently. Research has shown that environmental stresses can lead to oxidative stress in fish, usually shown as behavioral disorders, increasing amount of ROS, changes of enzyme activities, and organismal death. Liu (2006) reported that there was a positive correlation between the amount of ROS in the blood and the stocking densities of Chinese sturgeon. Qu *et al*. (2006) found the CAT activities in gills and muscles of tilapia exposed to phenol increased at first and then dropped in the groups with lower concentrations, while the CAT activities were controlled in the higher groups.

Since the TDG supersaturation was treated as a water quality issue, biological responses to TDG supersaturation in fish have been found. Newcomb (1974) mentioned that serum potassium and phosphate increased while serum albumin, calcium, and cholesterol decreased when the juvenile steelhead trout was exposed to nitrogen supersaturation. Liu *et al*. (2011) showed a trend that the CAT and SOD activities in gills and muscles of rock carp exposed to TDG levels ranging from 100% to 116% for 42 d increased first and then dropped.

The present study indicated that CAT activities in gills were higher than those in muscles, which agrees well with Liu *et al*. (2011). In this study the CAT activities in gills did not increase significantly in the treatment group. The reason may be that the CAT activities were induced briefly or the gills had already been damaged before they were increased significantly. In addition, there is a phenomenon that CAT activities in muscles were increased significantly. It indicated that the muscles were already affected by the TDG supersaturation. From then on, CAT activities began to drop. A possible explanation is that damage of the gills slowed down the respiration. The transport of TDG supersaturation to the inside of the body was blocked, and as a result the body was dying. These findings can provide a means to evaluate how seriously the fish are damaged by the TDG supersaturated water downstream from dams.

4.3 Suggestions

Since more and more high dams are being built or prepared in China, problems in reducing the TDG supersaturation need to be solved urgently. Qu *et al*., (2011) reported that the gas saturation downstream from a dam was associated with energy dissipation structures, spill rate, and operation patterns; the greater the ratio of spill rate vs. power flow was, the higher TDG supersaturation levels were generated. Orlins and Gulliver (2000) reported that TDG supersaturation could be substantially reduced through changing the spillway configurations equipped at the Wanapum dam. In order to reduce the gas saturation to a safe level, the water level can be lowered in advance to extend the flood discharge time and decrease the spill rate through the reservoir regulation, or choose surface or under-flow dissipation instead of ski-jump energy dissipation. The results of this study can facilitate future scheduling schemes for hydropower plants along the Yangtze River and help with the establishment of the water environment standards in China.

Acknowledgements

The authors are grateful to Mr. Wen-min YI, Mr. Xun-chi PU, Ms. Bo LI, Mr. Song WU, Mr. Zhi-guang ZHANG, Ms. Juan WEI, Mr. Zhong-luan YAN, Ms. Lu CAO, etc. (Sichuan University, China) for their help during the experiments.

References

Alderdice, D.F., Jensen, J.O.T., 1985. Assessment of the Influence of Gas Supersaturation on Salmonids in the Nechako River in Relation to Kemano Completion. Canadian Technical Report of Fisheries and Aquatic Sciences—1386, Canada Dept. of Fisheries and Oceans.

Arntzen, E.V., Geist, D.R., Murray, K.J., Vavrinec, J., Dawley, E.M., Schwartz, D.E., 2009. Influence of the hyporheic zone on supersaturated gas exposure to incubating chum salmon. *N. Am. J. Fish. Manage.*, **29**(6):1714-1727. [doi:10.1577/M08-212.1]

Beeman, J.W., Venditti, D.A., Morris, R.G., Gadomski, D.M., Adams, B.J., VanderKooi, S.P., Robinson, T.C., Maule, A.G., 2003. Gas Bubble Disease in Resident Fish below Grand Coulee Dam. Final Report of Research. Western Fisheries Research Center, Columbia River Research Laboratory, U.S. Geological Survey, Cook, WA.

Bohl, M., 1997. Gas bubble disease of fish. *Tierarzliche Prax. (German)*, **25**(3):284-288.

Chen, C.N., 2008. Research progress on Chinese sucker (*Myxocyprinus asiaticus* Bleeker) in China. *Fish. Sci. Technol. Inform.*, **35**(4):160-163 (in Chinese).

Gale, W.L., Maule, A.G., Postera, A., Peters, M.H., 2004. Acute exposure to gas-supersaturated water does not affect reproductive success of female adult chinook salmon late in maturation. *River Res. Appl.*, **20**(5):565-576. [doi:10.1002/rra.766]

Gray, R.H., Saroglia, M.G., Scarano, G., 1985. Comparative tolerance to gas supersaturated water of two marine fishes, *Dicentrarchus labrax* and *Mugil cephalus*. *Aquaculture*, **48**(1):83-89. [doi:10.1016/0044-8486(85)90054-7]

Huang, X., Li, K.F., Du, J., Li, R., 2010. Effects of gas supersaturation on lethality and avoidance responses in juvenile rock carp (*Procypris rabaudi* Tchang). *J. Zhejiang Univ.-Sci. B (Biomed. & Biotechnol.)*, **11**(10):806-811. [doi:10.1631/jzus.B1000006]

Jensen, J.O.T., Schnute, J., Alderdice, D.F., 1986. Assessing juvenile salmonid response to gas supersaturation using a general multivariate dose-response model. *Can. J. Fish. Aquatic Sci.*, **43**(9):1694-1709. [doi:10.1139/f86-213]

Li, R., Li, J., Li, K.F., Deng, Y., Feng, J.J., 2009. Prediction for supersaturated total dissolved gas in high-dam hydropower projects. *Sci. China Series E: Technol. Sci.*, **52**(12):3661-3667. [doi:10.1007/s11431-009-0337-4]

Liu, S.Y., 2006. Effect of Environmental Stress on Free Radical Level and Antioxidant Enzymes Activities of Chinese Sturgeon, Acipenser Sinensis. MS Thesis, Huazhong Agricultural University, Wuhan, China (in Chinese).

Liu, X.Q., Li, K.F., Du, J., Li, J., Li, R., 2011. Growth rate, catalase and superoxide dismutase activities in rock carp (*Procypris rabaudi* Tchang) exposed to supersaturated total dissolved gas. *J. Zhejiang Univ.-Sci. B (Biomed. & Biotechnol.)*, **12**(11):909-914. [doi:10.1631/jzus.B1100071]

Montavon, P., Kukic, K.R., Bortlik, K., 2007. A simple method to measure effective catalase activities: optimization, validation, and application in green coffee. *Anal. Biochem.*, **360**(2):207-215. [doi:10.1016/j.ab.2006.10.035]

Nebeker, A.V., Andros, J.D., McCrady, J.K., Stevens, D.G., 1978. Survival of steelhead trout (*Salmo gairdneri*) eggs, embryos, and fry in air-supersaturated water. *J. Fish. Res. Board Can.*, **35**(2):261-264. [doi:10.1139/f78-043]

Newcomb, T.W., 1974. Changes in blood chemistry of juvenile steelhead trout, salmo gairdneri, following sublethal exposure to nitrogen supersaturation. *J. Fish. Res. Board Can.*, **31**(12):1953-1957. [doi:10.1139/f74-257]

Orlins, J.J., Gulliver, J.S., 2000. Dissolved gas supersaturation downstream of a spillway. II: computational model. *J. Hydraulic Res.*, **38**(2):151-159. [doi:10.1080/00221680009498350]

Qu, J.H., Chen, J.Z., Hu, G.D., Wu, W., 2006. Dynamic changes of catalase and glutathione-*S*-transferase in the different tissues of tilapia exposed to phenol. *Ecol. Environ.*, **15**(4):687-692 (in Chinese).

Qu, L., Li, R., Li, J., Li, K.F., Deng, Y., 2011. Field observation of total dissolved gas supersaturation of high-dams. *Sci. China Technol. Sci.*, **54**(1):156-162. [doi:10.1007/s11431-010-4217-8]

Stevens, D.G., Nebeker, A.V., Baker, R.J., 1980. Avoidance responses of salmon and trout to air-supersaturated water. *Trans. Am. Fish. Soc.*, **109**(6):751-754. [doi:10.1577/1548-8659(1980)109<751:AROSAT>2.0.CO;2]

Tan, D.C., 2006. Research on the Lethal Effect of the Dissolved Gas Super-saturation Resulted from Thee Gorges Project to Fish. MS Thesis, Southwest University, Chongqing, China (in Chinese).

Wang, S.T., He, X.J., An, R.D., 2010. Responses of growth and antioxidant metabolism to nickel toxicity in Luffa cylindrical seedlings. *J. Anim. Plant Sci.*, **7**(2):810-821.

Zhou, Y.X., Zhang, Z.H., 1989. Method of Toxicity Tests on Aquatic Creatures. Agriculture Press, Beijing, China, p.109-122 (in Chinese).

Recommended paper related to this topic

Growth rate, catalase and superoxide dismutase activities in rock carp (*Procypris rabaudi* Tchang) exposed to supersaturated total dissolved gas

Authors: Xiao-qing LIU, Ke-feng LI, Jun DU, Jia LI, Ran LI
doi:10.1631/jzus.B1100071
J. Zhejiang Univ.-Sci. B (Biomed. & Biotechnol.), 2011 Vol.12 No.11 P.909-914

Abstract: Total dissolved gas supersaturation (TDGS) appears when the pressures of gases in a solution exceed the barometric pressures. TDGS is often caused by flood discharge at dams. It may lead to gas bubble disease (GBD) for fish and biochemical responses of selected fish and other aquatic organisms. The purpose of this study was to determine the impact of long-term TDGS levels on the growth and biochemical responses of rock carp (*Procypris rabaudi* Tchang) dwelling in the upper reaches of the Yangtze River. Three-year-old rock carp were exposed to TDGS levels at 100%, 104%, 108%, 112%, and 116% for 42 d. Samples were taken every 7 d after the start of the trial in order to determine catalase (CAT) and superoxide dismutase (SOD) activities in gill and muscle tissues. Samples were taken at Days 0 and 42 of exposure to determine growth rate. Little effect was found on growth rate in all treatment groups. SOD and CAT activities varied in different tissues, according to time of exposure and TDGS levels. The biochemical response of fish exposed to TDGS was more obvious in gill tissue than in muscle tissue. Surveys of SOD and CAT activities in different tissues offer important information about the effect of TDGS on the rare fish in the Yangtze River, and may help evaluate the risk to the aquatic eco-environment and aquatic ecosystem in the downstream of the Yangtze River.