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

Effect of total dissolved gas supersaturated water on early life of David's schizothoracin (*Schizothorax davidi*)*

Rui-feng LIANG[1], Bo LI[1,2], Ke-feng LI[†‡1], You-cai TUO[1]

([1]*State Key Laboratory of Hydraulics and Mountain River Engineering, Sichuan University, Chengdu 610065, China*)

([2]*Changjiang River Scientific Research Institute, Wuhan 430010, China*)

[†]E-mail: kefengli@scu.edu.cn

Received Dec. 29, 2012; Revision accepted May 4, 2013; Crosschecked June 5, 2013

Abstract: The effect of total dissolved gas (TDG) supersaturation on fish living downstream of dams is one of the main ecological risks of high dam construction. A strategy for mitigating the negative effects is needed urgently since many high dams are under construction in the upper reaches of the Yangtze River in China. Experiments on the hatching process of David's schizothoracin were carried out and the results show that the hatching rate decreased with increasing TDG levels, and that most eggs hatched within a very short time in the higher TDG saturation groups. By using a stereomicroscope, damages to the head, yolk sac, body, anus, etc. were found in larvae which hatched in TDG supersaturated water. Results show that the lesion rate increased with increasing TDG levels. Furthermore, 7-d-old David's schizothoracin were exposed to TDG supersaturated water levels of 100%, 105%, 110%, 115%, 120%, 125%, 130%, 135%, and 140% for testing their tolerance to TDG supersaturation. We found that the median lethal concentrations (LC_{50}) for 13, 14, 20, 35, 52, 73, and 96 h exposure were 138%, 138%, 134%, 130%, 129%, 128%, and 126%, respectively. The median lethal times (LT_{50}) were 7.49, 11.04, 19.25, and 35.38 h for exposure to water with TDG levels of 145%, 140%, 135%, and 130%, respectively.

Key words: Total dissolved gas (TDG), TDG supersaturation, David's schizothoracin, Hatch, Larvae
doi:10.1631/jzus.B1200364 **Document code:** A **CLC number:** Q17

1 Introduction

When water spills over a dam and falls into the tailrace, it can be supersaturated with atmospheric air. The effect of total dissolved gas (TDG) supersaturation on fish downstream of dams has become one of the most important ecological risks of high dam projects. TDG saturation levels greater than 120% were observed downstream from most dams in the Columbia River, USA and its branches during dam spilling (Ryan and Dawley, 1998; US Army Corps of Engineer, 2005). Li *et al*. (2009) reported that in China, TDG levels had reached 140%, 130%, 124%, and 143% downstream from the Ertan Dam, Zipingpu Dam, Manwan Dam, and Three Gorges Dam, respectively. TDG supersaturation was still found hundreds of meters downstream from these dams. Many studies have concluded that TDG supersaturated water, with saturation levels ranging from 105% to 110%, can cause gas bubble disease (GBD) in fish. Fish can die within a few hours when they are exposed to TDG levels greater than 140% (Backman and Evans, 2002; Weitkamp *et al*., 2003).

Many studies have reported that tolerance to TDG supersaturation varies among different fish species, stages of development, and fish of different sizes (Bentley *et al*., 1976; Bouck, 1976; Beeman *et al*., 2003). There are two opposite opinions about the tolerance of fish at different developmental stages. Some studies suggest that tolerance increases with the life stages and the most serious damage is likely to occur in larvae and fry (Dennison and Marchyshyn,

‡ Corresponding author
* Project (No. 50979063) supported by the National Natural Science Foundation of China

1973; Cornacchia and Colt, 1984), but others found that tolerance of fish to gas supersaturation decreases from the alevin stage to the juvenile stage (Weitkamp and Katz, 1980; Krise and Herman, 1989).

Studies on the effects of TDG supersaturation on fish started late in China. Tan *et al*. (2006) explored the tolerance and adaptive behavior of fish in TDG supersaturated water. Xu *et al*. (2008) found that growth and food intake of turbot were promoted by oxygen supersaturation. Acute lethal and avoidance response experiments on rock carp exposed to TDG supersaturation were carried out by Huang *et al*. (2010a). Liu *et al*. (2011) studied the sensitivity of different tissues of rock carp of different ages to TDG supersaturated water. They found that gill tissue was more sensitive than muscle, and that three-year-old fish were the most sensitive while one-year-old fish were the least. In China, the time for most fish spawning and hatching in the Yangtze River coincides with dam spilling periods. Few studies have been made of the effects of TDG supersaturation on the early life and the physiological activity of fish. In this study, David's schizothoracin living in the upper reaches of the Yangtze River and seriously threatened by TDG supersaturation were chosen as the study's objects. Symptoms of gas bubble disease, the lesion rate, the median lethal concentration (LC_{50}), and the median lethal time (LT_{50}) were observed or calculated to determine the effect of TDG supersaturation on David's schizothoracin. These experiments may be helpful for establishing water quality standards for hydropower projects in China, and for evaluating the effects of TDG supersaturation on the ecosystem.

2 Materials and methods

2.1 Experimental materials and instrumentations

A hatching experiment was carried out with David's schizothoracin eggs, which were obtained from the Lusan Baojian Fishery Company, Ya'an, Sichuan, China. Before the trial, eggs were kept in a tank at the State Key Laboratory of Hydraulics and Mountain River Engineering (Sichuan University, China) for 27 h, where the water temperature was (20±0.5) °C, and the pH value 7.3±0.3 (mean± standard deviation (SD)). A tolerance test was conducted for 7-d-old larvae hatching in normal conditions. During the experiment, water temperature, TDG levels, dissolved oxygen (DO), and pH values were measured daily using a mercury thermometer, Point Four tracker (Point Four Systems Inc., Canada), dissolved oxygen meters (Oxi 3210 SET 3 Inc., Germany), and a digital pH meter (JENCO Model 6010, China), respectively. The experimental conditions were as follows: water temperature (20±0.5) °C, pH 7.3±0.3, DO 7.5–8.0 mg/L.

2.2 Effect of TDG supersaturation on the hatching of David's schizothoracin

An experiment was carried out using the system available at the State Key Laboratory of Hydraulics and Mountain River Engineering. TDG supersaturated water can be generated by pouring air and water into an autoclave at the same time (Huang *et al*., 2010b). Parallel tests were carried out. Fifty eggs were held in each of a series of tanks with saturation levels of 100%, 105%, 110%, 115%, 120%, 125%, 130%, 135%, and 140%. During the experiment, the hatching time was recorded and the damage to the fish was observed with a stereomicroscope. The hatching rate, median hatching time (HT_{50}), and lesion rate were calculated.

2.3 Study on the tolerance of David's schizothoracin to TDG supersaturation

Twenty 7-d-old larvae hatching in normal conditions were exposed to each of a series of tanks with TDG levels of 100%, 105%, 110%, 115%, 120%, 125%, 130%, 135%, 140%, and 145%, respectively. Parallel tests were carried out under the same conditions. In the experiment, the behavior of fish was observed continuously, and the time of death of each fish was recorded. According to the results, the LT_{50} was determined using Probit method, and the LC_{50} was calculated by linear interpolation (Zhou and Zhang, 1989).

3 Results

3.1 Effects of TDG supersaturation on the hatching of David's schizothoracin

3.1.1 Hatching rate and time

Fig. 1 shows that the relationship between hatching rate and TDG supersaturation could be approximated by a linear equation, $y=-0.7333x+164.44$ ($R^2=0.9624$), where y is the hatching rate, x is the TDG saturation level, and R^2 is the related coefficient.

Fig. 1 indicates that the hatching rate decreased with increasing TDG levels. The hatching time of David's schizothoracin was affected even at low saturations such as 105% and 110%, and was seriously affected in groups with high TDG levels of 135% and 140%.

In Fig. 2, the relationship between the HT_{50} and the TDG supersaturation can be expressed by $y=0.0072x^2-1.6367x+138.03$ ($R^2=0.9338$), where y is the HT_{50}, x is the TDG saturation level, and R^2 is the related coefficient.

The relationship between the TDG supersaturation and the time for all larvae to hatch (HT_{100}) could be approximated by $y=0.0058x^2-1.6126x+162.72$ ($R^2=0.9828$), where y is the HT_{100}, x is the TDG saturation level, and R^2 is the related coefficient.

There was little influence on the HT_{50} in the lower TDG groups, but as TDG levels increased, the HT_{50} began to rise, and HT_{100} was negatively correlated with the TDG supersaturation. Most eggs hatched in a very short time in higher TDG supersaturated water, as shown by the HT_{50} and HT_{100} values, which is not beneficial for their survival in nature due to competition for food.

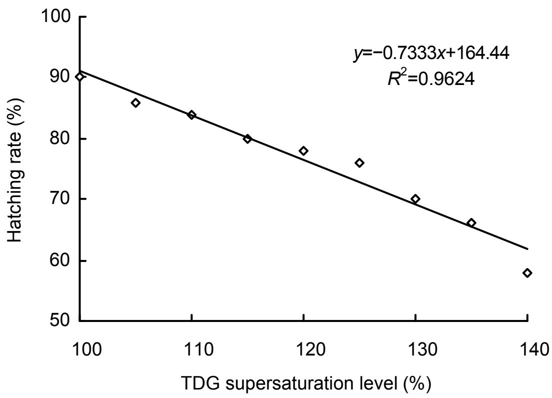

Fig. 1 Relationship between hatching rates and TDG supersaturation levels

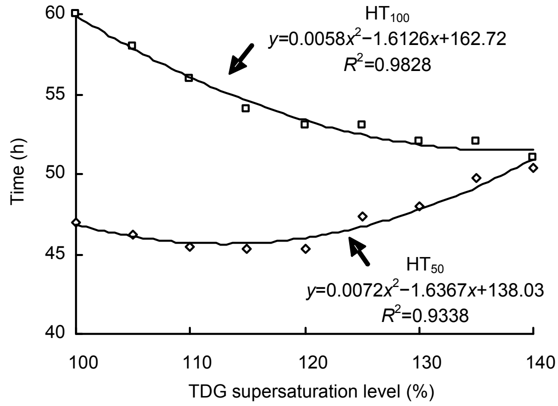

Fig. 2 Relationships between HT_{50}, HT_{100}, and TDG supersaturation levels

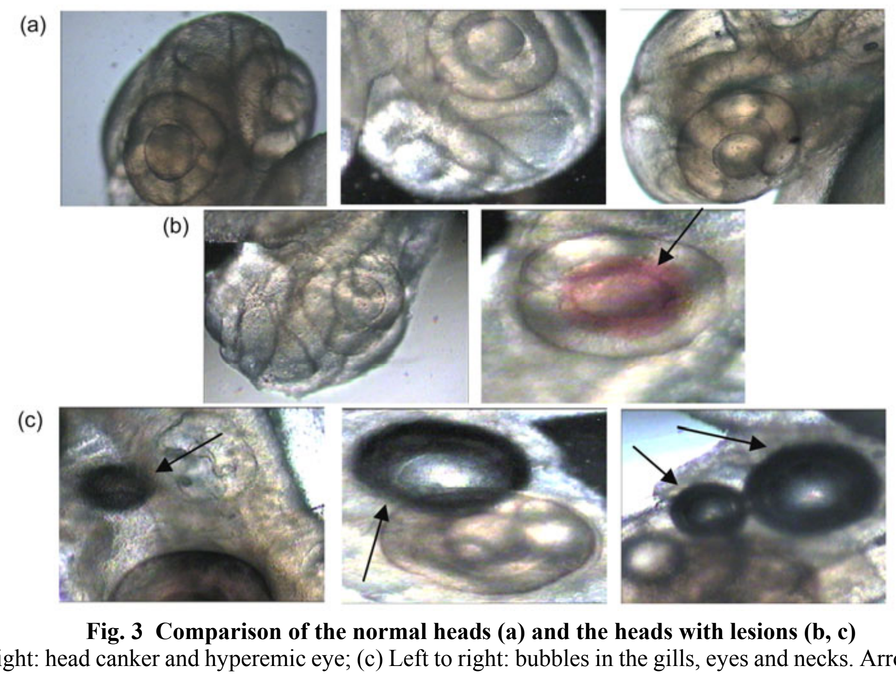

Fig. 3 Comparison of the normal heads (a) and the heads with lesions (b, c)

(b) Left to right: head canker and hyperemic eye; (c) Left to right: bubbles in the gills, eyes and necks. Arrows point out the parts with lesions

3.1.2 Lesion rate

During the experiment, eggs in the treatment groups were floating on the water surface with lots of bubbles around them and could not sink to the bottom of the tanks. The larvae were examined with a stereomicroscope to explore the effects of TDG supersaturation on the hatching of David's schizothoracin, and the lesion rates were calculated.

Figs. 3–8 show that the hatching of David's schizothoracin was seriously affected by TDG supersaturation. Lesions were found in several parts of the fish, such as the eyes, head, and tail. Fig. 9 shows that the lesion rate increased with the TDG level. Owing to random sampling, sometimes lesion rates in the lower TDG groups were higher than those in other groups, such as the rates at TDG levels of 110% and 115% in Sample 2 (Fig. 9b).

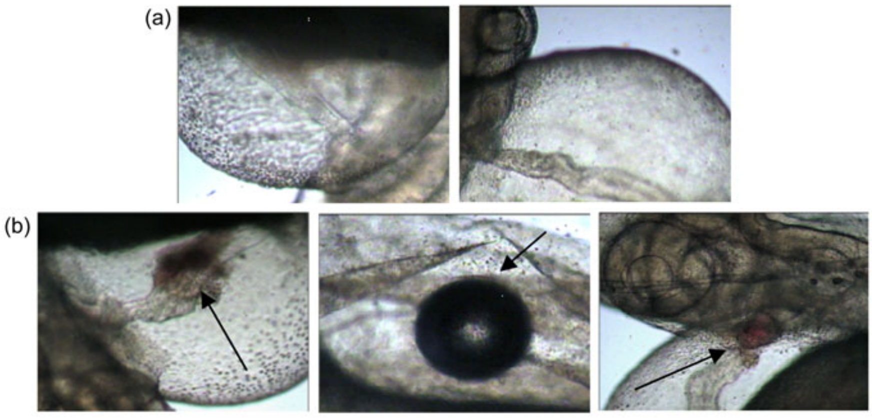

Fig. 4 Comparison of the normal yolk sacs (a) and the yolk sacs with lesions (b)
(b) Left to right: rots in the nutrient canal, bubbles in the yolk sac and yolk sac congestion. Arrows point out the parts with lesions

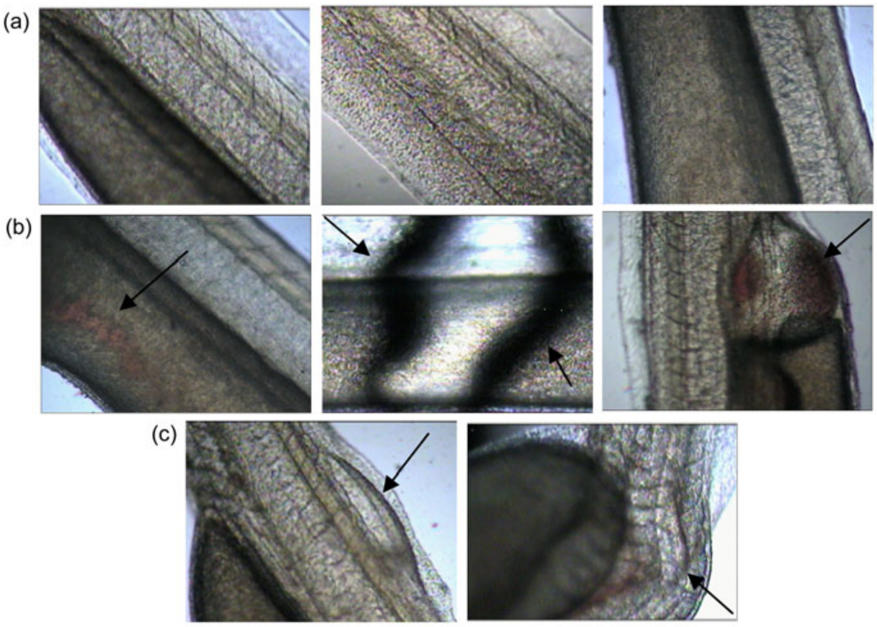

Fig. 5 Comparison of the normal bodies (a) and the bodies with lesions (b, c)
(b) Left to right: body congestion, bubbles in the body, and air embolus in the body; (c) Left to right: body swelling and body bent. Arrows point out the parts with lesions

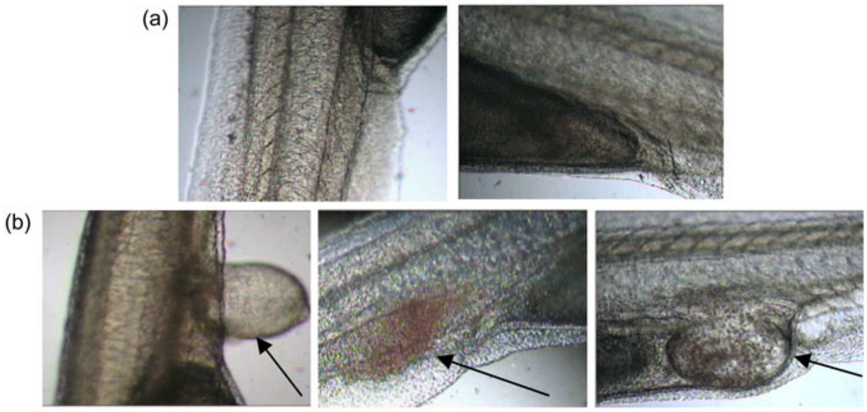

Fig. 6 Comparison of the normal anuses (a) and the anuses with lesions (b)
(b) Left to right: swelling, congestion, and air embolus in the anus. Arrows point out the parts with lesions

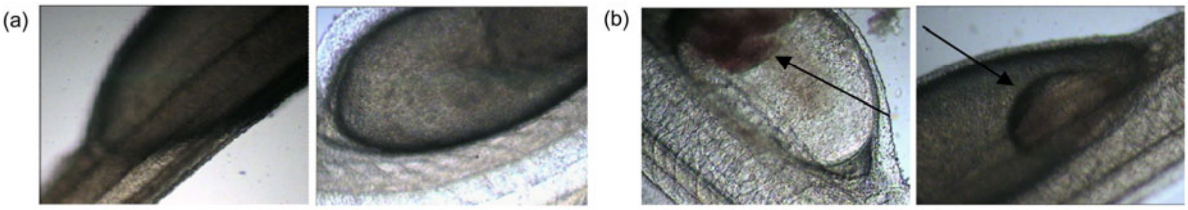

Fig. 7 Comparison of the normal fish bellies (a) and the bellies with lesions (b)
(b) Left to right: congestion and bubble in the fish bellies. Arrows point out the parts with lesions

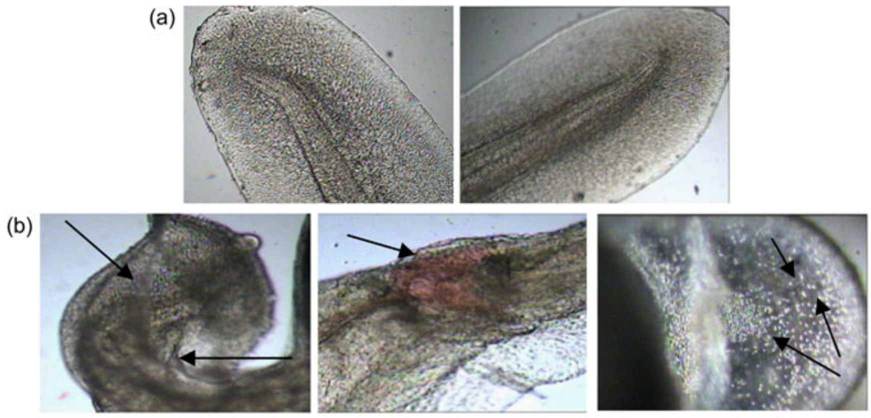

Fig. 8 Comparison of the normal fish tails (a) and the tails with lesions (b)
(b) Left to right: tails bent, congestion, and bubbles in the tails. Arrows point out the parts with lesions

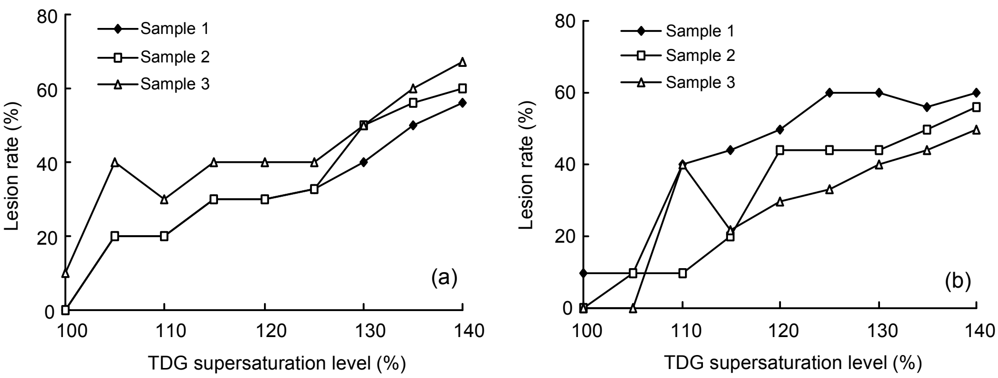

Fig. 9 Relationship between the lesion rates and TDG supersaturation levels
(a) One day after hatching; (b) Two days after hatching

3.2 Acute lethality experiment

Figs. 10 and 11 show the LT_{50} at different TDG supersaturations and the LC_{50} at different times. Larvae died a few hours after exposure in the water to TDG levels greater than 135%, and the LT_{50} increased dramatically at 130% saturation and after 96 h of exposure. About 30% and 20% dead fish were found in the 125% and 120% saturation tanks, respectively. Almost no dead fish were found in the 100%, 105%, 110%, and 115% saturation tanks, so the LT_{50} was not calculated in these cases.

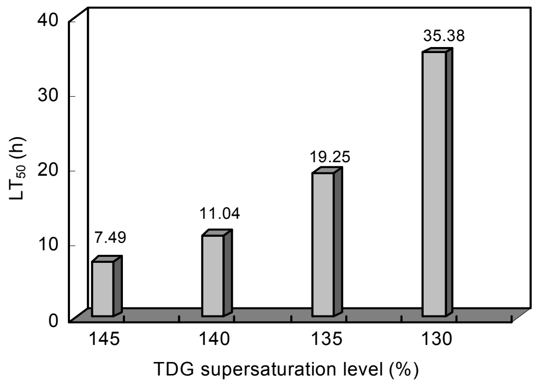

Fig. 10 LT_{50} for David's schizothoracin at different TDG supersaturation levels

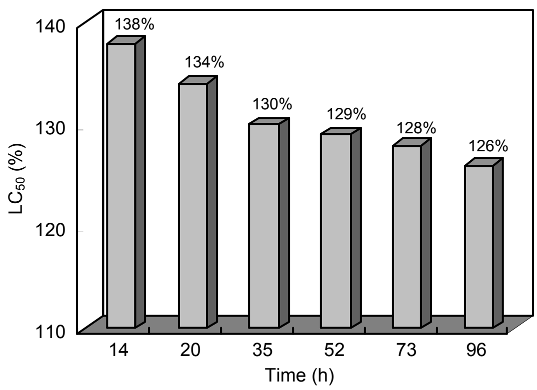

Fig. 11 LC_{50} for David's schizothoracin at different times

4 Discussion

4.1 Hatching experiment

It is essential for embryos to develop during the period from the fertilized egg to the pre-hatching stage. Development may terminate or embryos may suffer a higher lesion rate and a lower hatching rate by hatching prematurely from the fertilized egg. In the present study, the HT_{50} increased with increasing TDG levels, the hatching time became much more condensed, the hatching rate decreased, and the lesion rate increased in the high TDG saturation groups. In such cases, we conclude that the early life of David's schizothoracin could be seriously affected by TDG supersaturation.

Many studies have been carried out on the early life of fish. The hatching and survival of a fish can be affected by several factors.

Alderdice and Forrester (1971) found that cod eggs could incubate successfully when the water temperature ranged from 2.5 °C to 8.5 °C, and determined some limiting temperatures in different situations during the spawning period. Guo (1982) reported that water temperatures above 5 °C were harmful to the hatching of grass carp. Experimental results showed that a water temperature of 12 °C was suitable for egg incubation, while yolk absorption was most efficient at 15 °C (Hart and Purser, 1995). In this study, the water temperature was kept stable at 20 °C, a temperature suitable for hatching of David's schizothoracin.

Rombough (1988) and Oppen-Berntsen *et al.* (1990) reported that hatching could be delayed in a hyperoxic environment and advanced under a hypoxic environment. DO levels increased as TDG saturation increased, and the hatching time was longer in tanks with higher TDG levels. Thus, our results agree well with theirs.

Noor-Hamid *et al.* (1994) reported that fish larvae could easily be damaged by decreasing pH values. Lee and Menu (1981) and Swanson (1996) pointed out that the development of eggs can be affected by salinity. Other studies suggested that light intensity and pollutants could also trouble the hatching process (Shan, 1993; Chai *et al.*, 2008).

Since the hatching period is affected by so many factors, keeping a stable experiment is of great importance. The effects of different combinations of these factors on hatching need further study. The present study provides important information concerning the effects of TDG supersaturation on fish in the Yangtze River.

4.2 Acute lethality experiment

Similar to the effects on hatching, the tolerance of fish was also affected by many factors, including water temperature, life stage, species, and TDG saturation levels.

Nebeker *et al.* (1978) found that the mortality of rainbow trout, but not of red salmon, increased with temperature. As in the hatching experiment, a stable water temperature was very important for tolerance.

Bouck (1976) reported that adult trout died nearly 200 h later than juvenile fish when the mortality reached 20%. Arntzen *et al*. (2009) found that chum salmon eggs showed a strong tolerance to TDG levels from 110% to 115%. Jensen *et al*. (1986) reported that fish in high TDG levels died quicker than those in lower levels. The present results match well those of Jensen *et al.* (1986) and Huang *et al.* (2010a).

Beeman *et al*. (2003) found that the LT_{50} differed among fish species (Table 1). Results in this study showed that the LT_{50} of larvae of David's schizothoracin exposed to TDG levels of 130% was 35.4 h. The larvae showed a relatively high tolerance to TDG supersaturation compared to the other fish species listed in Table 1.

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

Species	LT_{50} (h)	
	125% TDG	130% TDG
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
David's schizothoracin		35.4

* Data are from Beeman *et al*. (2003) and the present study (David's schizothoracin)

Therefore, to set up water quality standards in China, the responses of different fish species at different stages of development in conditions close to the natural environment need to be studied. However, the present results provide some basic data for establishing water quality standards in China.

5 Conclusions

The effects of TDG supersaturation on the early life of David's schizothoracin were studied. The conclusions drawn from this study can be summarized as follows:

1. The hatching rate was negatively correlated to the TDG supersaturation, and the hatching time became more condensed as TDG supersaturation increased.

2. Lesions, such as congestion bubbles, were found in the heads, eyes, and tails of fish hatched in TDG supersaturated water. The lesion rate was positively related to the TDG supersaturation.

3. The threshold of TDG supersaturation for 7-d-old David's schizothoracin could be set at 125% under these experimental conditions.

Compliance with ethics guidelines

Rui-feng LIANG, Bo LI, Ke-feng LI, and You-cai TUO declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

References

Alderdice, D.F., Forrester, C.R., 1971. Effects of salinity, temperature, and dissolved oxygen on early development of the pacific cod (*Gadus macrocephalus*). *J. Fish. Res. Board Can.*, **28**(6):883-902. [doi:10.1139/f71-130]

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]

Backman, T.W.H., Evans, A.F., 2002. Gas bubble trauma incidence in adult salmonids in the Columbia River Basin. *N. Am. J. Fish. Manage.*, **22**(2):579-584. [doi:10.1577/1548-8675(2002)022<0579:GBTIIA>2.0.CO;2]

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, US Geological Survey, Cook, WA.

Bentley, W.W., Dawley, E.M., Newcomb, T.W., 1976. Some Effects of Excess Dissolved Gas on Squawfish, *Ptychocheilus Oregonensis* (Richardson). *In*: Gas Bubble Disease. Technical Information Center, Oak Ridge, Tennessee, p.41-46.

Bouck, G.R., 1976. Supersaturation and Fishery Observations in Selected Alpine Oregon Streams. *In*: Gas Bubble Disease. Technical Information Center, Oak Ridge, Tennessee, p.37-40.

Chai, Y., Xie, C.X., Wei, Q.W., Li, L.X., 2008. Effect of different water depths and light intensity on the hatching of Chinese sturgeon. *Water Conserv. Relat. Fish.*, **28**(3):32-33 (in Chinese).

Cornacchia, J.W., Colt, J.E., 1984. The effects of dissolved gas supersaturation on larval striped bass, *Morone saxatilis* (Walbaum). *J. Fish Dis.*, **7**(1):15-27. [doi:10.1111/j.1365-2761.1984.tb00903.x]

Dennison, A.B., Marchyshyn, M.J., 1973. A device for alleviating supersaturation of gases in hatchery water supplies. *Progressive Fish-Culturist*, **35**(1):55-58. [doi:10.1577/1548-8659(1973)35[55:ADFASO]2.0.CO;2]

Guo, Y.C., 1982. Effects of water temperature on the embryonic development of hypophthalmichthys molitrix and *Ctenopharyngodon idellus*. *Freshwater Fisheries*, (3): 35-40 (in Chinese).

Hart, R.P., Purser, G.J., 1995. Effects of salinity and temperature on eggs and yolk sac larvae of the greenback flounder (*Rhombosolea tapirina* Günther, 1862). *Aquaculture*, **136**(3-4):221-230. [doi:10.1016/0044-8486(95)01061-0]

Huang, X., Li, K.F., Du, J., Li, R., 2010a. 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]

Huang, X., Li, K.F., Li, R., Li, J., Du, J., 2010b. Experimental system for the simulation of total dissolved gas supersaturated water of high dams. *J. Sichuan Univ. (Eng. Sci. Ed.)*, **42**(4):25-28 (in Chinese).

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. Aquat. Sci.*, **43**(9):1694-1709. [doi:10.1139/f86-213]

Krise, W.F., Herman, R.L., 1989. Tolerance of lake trout, (*Salvelinus namaycush* Walbaum), sac fry to dissolved gas supersaturation. *J. Fish Dis.*, **12**(3):269-273. [doi:10.1111/j.1365-2761.1989.tb00312.x]

Lee, C., Menu, B., 1981. Effects of salinity on egg development and hatching in grey mullet (*Mugil cephalus* L.). *J. Fish Biol.*, **19**(2):179-188. [doi:10.1111/j.1095-8649.1981.tb05822.x]

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, 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]

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]

Noor-Hamid, S., Fortes, D.R., Parado-Estepa, F., 1994. Effect of pH and ammonia on survival and growth of the early larval stages of *Penaeus monodon* Fabricius. *Aquaculture*, **125**(1):67-72. [doi:10.1016/0044-8486(94)90283-6]

Oppen-Berntsen, D.O., Bogsnes, A., Walther, B.T., 1990. The effects of hypoxia, alkalinity and neurochemicals on hatching of *Atlantic salmon* (*Salmo salar*) eggs. *Aquaculture*, **86**(4):417-430. [doi:10.1016/0044-8486(90)90330-P]

Rombough, P.J., 1988. Growth, aerobic metabolism, and dissolved oxygen requirements of embryos and alevins of steelhead, *Salmo gairdneri*. *Can. J. Zool.*, **66**(3):651-660. [doi:10.1139/z88-097]

Ryan, B.A., Dawley, E.M., 1998. Effects of Dissolved Gas Supersaturation on Fish Residing in the Snake and Columbia Rivers, 1997. Unpublished Report. National Oceanic and Atmospheric Administration to Bonneville Power Administration, Portland Oregon, p.60.

Shan, S.Y., 1993. Effects of sewage in benxi section of Taizi river on the early life of fish. *Carcinogen. Teratogen. Mutagen.*, **5**(6):63-64 (in Chinese).

Swanson, C., 1996. Early development of milkfish: effects of salinity on embryonic and larval metabolism, yolk absorption and growth. *J. Fish Biol.*, **48**(3):405-421. [doi:10.1111/j.1095-8649.1996.tb01436.x]

Tan, D.C., Ni, Z.H., Zheng, Y.H., Li, L.Y., Li, Y.F., 2006. Dissolved gas supersaturation downstream of dam and its effects on fish. *Freshwater Fisheries*, **36**(3):56-59 (in Chinese).

US Army Corps of Engineer, 2005. Libby Dam January 2005 Relay Maintenance Operations Total Dissolved Gas Monitoring Study. US Army Corps of Engineer.

Weitkamp, D.E., Katz, M., 1980. A review of dissolved-gas supersaturation literature. *Trans. Am. Fish. Soc.*, **109**(6): 659-702. [doi:10.1577/1548-8659(1980)109<659:ARODGS>2.0.CO;2]

Weitkamp, D.E., Sullivan, R.D., Swant, T., DosSantos, J., 2003. Gas bubble disease in resident fish of the Lower Clark Fork River. *Trans. Am. Fish. Soc.*, **132**(5):865-876. [doi:10.1577/T02-026]

Xu, Y., Qu, K.M., Ma, S.S., 2008. Effects of hyperoxic dissolved oxygen levels on growth and digestive enzyme of turbot, *Scophthalmus maximus*. *Fish. Mod.*, **35**(4):24-27 (in Chinese).

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