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Effects of gas supersaturation on lethality and avoidance responses in juvenile rock carp (*Procypris rabaudi* Tchang)*

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Abstract: Laboratory experiments were conducted to determine the effects of total dissolved gas (TDG) supersaturation on acute lethality and avoidance responses in juvenile rock carp (*Procypris rabaudi* Tchang). The juvenile rock carp were exposed to water with different levels of supersaturation (105%, 115%, 120%, 125%, 130%, 135%, 140%, and 145%) and depth of 0.20 m at 25 °C for 60 h. Median lethal time (LT_{50}) was used to assess the lethal responses corresponding to different levels of gas supersaturation. The results show that half of the juvenile rock carp died at the 120%, 125%, 130%, 135%, 140%, and 145% levels of supersaturation, and the LT_{50} corresponding to different levels of supersaturation was 18.7, 15.4, 8.2, 6.6, 3.5, and 1.7 h. When the level of supersaturated water is below 115%, the mortality is negligible. Avoidance responses were observed 5 min after the fish were put into equilibrated water (99%, 0.08 m deep) and water with different supersaturated levels (105%, 115%, 125%, 135%, and 145%, 0.08 m deep) at 25 °C. The fish exhibited strong avoidance responses in supersaturated water when the gas supersaturation was above 135%. However, they exhibited an obvious preference to supersaturated water when the gas supersaturation was below 115%. Thus, the juvenile rock carp can likely survive in water with a supersaturated level of 115%.

Key words: Gas supersaturation, Lethality, Avoidance, Juvenile rock carp (*Procypris rabaudi* Tchang)
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1 Introduction

Total dissolved gas (TDG) supersaturation occurs when the partial pressures of atmospheric gases in solution exceed the respective partial pressures in atmosphere. TDG supersaturation downstream from a dam generates from flood discharge. Previous studies have reported that salmonids have died from gas bubble disease (GBD) when the gas saturation level was in range of 120% to 143% in the Columbia River (Ebel, 1969; Beiningen and Ebel, 1970; 1971; Meekin and Turner, 1974).

McGrath *et al*. (2006) provided a review of literature relevant to the Columbia River system and suggested that a TDG lower than 120% may detrimentally affect sensitive species and the life stages of fishes as well as other organisms. National Oceanic and Atmospheric Administration (NOAA, 1995; 2000) indicated that TDG supersaturation levels between 110% and 120% have minimal impacts on aquatic biota in river environment. Therefore, a 120% TDG saturation in the tail water downstream from the dam and 115% TDG saturation in the fore bays have been allowed in Oregon and Washington States (NOAA, 1995).

Though the effect of TDG supersaturation on organisms has been acknowledged as an

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environmental concern, many countries have not set a limit for gas supersaturation, and this is especially true for China. During flood discharging in 2006 and 2007, TDG supersaturation downstream from the Three Gorges Dam exceeded 130% for several days (Li *et al*., 2009), and fish died in the Gezhouba reservoir due to GBD (Tan, 2006). Few studies are available on the lethality and avoidance responses in domestic species that were exposed to TDG supersaturated water in the Yangtze River. The objectives of this study are to determine the effects of TDG supersaturation on acute lethality in juvenile rock carp (*Procypris rabaudi* Tchang) and to test the lateral avoidance responses in juvenile rock carp within various levels of supersaturated water.

2 Materials and methods

2.1 Collection and holding of experimental fish

Juvenile rock carp (*Procypris rabaudi* Tchang) which inhabit the upstream region of the Yangtze River were chosen for the experiment. They were hatched on April 23, 2009 from the Sichuan Fisheries Research Institute and transferred to the laboratory on July 3, 2009. The juveniles were placed in the laboratory for a fortnight to acclimate to living conditions, and it was assured that the mortality was less than 5% before initial testing. The experimental conditions were as follows: water temperature 25 °C, salinity 20‰, dissolved oxygen (DO) 7.5–8.0 mg/L, day/night-cycle 14 h light:10 h dark. The fish were fed on *Limnodrilus hoffmeisteri* once a day, and there was no feeding during the testing. The fish weight ranged from 2.41 to 2.48 g, while the total length ranged from 6.6 to 7.2 cm.

2.2 Water quality monitoring

A Point-Four device (Point Four Systems Inc., Canada) was used to monitor TDG (%), temperature (°C), barometric pressure (mmHg), and Δ*p* (differential pressure). A YSI 6600 device (YSI Inc., USA) was used to regularly monitor oxygen (%), temperature (°C), pH, and salinity (‰). Data were automatically monitored once a minute during the experiments.

During experiments, the probe of the Point Four was agitated gently by hand every 5 min to dislodge air bubbles which were attached by TDG sensor membranes until the display reading data were stable. The gas saturations were measured and verified every hour.

2.3 Experimental design

2.3.1 Temperature control and supersaturated water generation

The system (Huang *et al*., 2010) can generate water with desired gas saturation by manipulating the combination of water inflow, water outflow, water temperature, and air pressure of the pressure vessel. It is realistic to be able to keep the water temperature and gas saturation constant for 60 h during the experiments. The schematic of the experimental system is shown in Fig. 1.

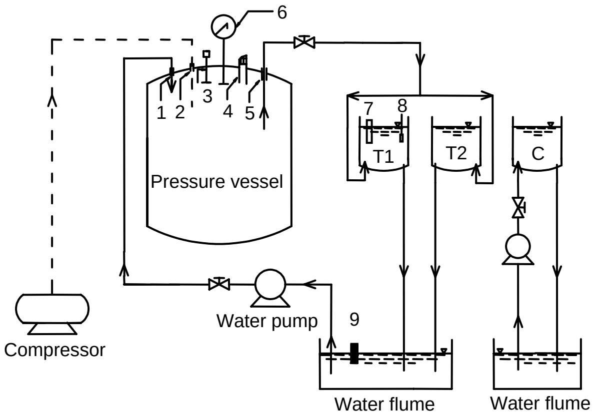

Fig. 1 Schematic of an experimental system
1: water inlet; 2: gas inlet; 3: pressure vacuum valve; 4: safety valve; 5: water outlet; 6: pressure gauge; 7: YSI 6600; 8: Point 4; 9: temperature control device. T1: treatment group 1; T2: treatment group 2; C: control group

Water temperature was controlled by an electric device composed of a heater and a sensor controlling the water temperature in the water flume. The heater was turned on when the water temperature monitored by the sensor was lower than the designated one, and the heater was turned off when the water temperature was higher than the designated one.

Temperature-controlled water was supplied to the water pump and passed through the pump into the pressure vessel, and air was injected into the vessel by a compressor at the same time. The TDG supersaturated water was generated by adding pressure to the water, allowing achievement of desired TDG saturation levels by regulating the water inflow, water outflow, and air pressure of the pressure vessel. Then the supersaturated water flowed into the treatment groups (T1, T2) and flowed back to the water flume by gravity. The supersaturated water was a circulation of water flow and the inflow water pressure was maintained by using the water pump. The methods of the control of the water temperature and the circulation of water flow were the same as the supersaturated water supply.

2.3.2 Acute lethal experiments

Three identical Plexiglas containers were used for aquaria. The depth of the container was 1.2 m and the diameter 0.4 m. There are two holes with diameter of 0.02 m at the bottom for water inlet and outlet (Fig. 2). A plastic mesh net is attached to the hole as a shroud to keep the fish in the container. The experimental circumstance was similar to the acclimated one. The water depth was kept for 0.2 m by controlling the balance of inflow and outflow at 0.1 L/s.

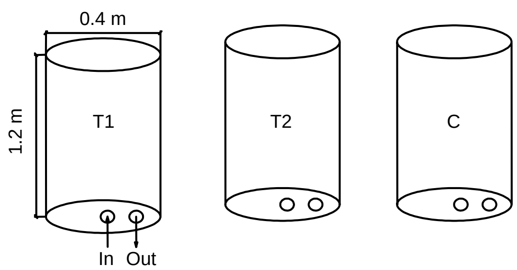

Fig. 2 Front view of containers used in lethal experiments
The surface is free-surface flow. T1: treatment group 1; T2: treatment group 2; C: control group

Different levels of supersaturated water were conducted (105%, 115%, 120%, 125%, 130%, 135%, 140%, and 145%) into the treatment groups and the equilibrated water (99%) was conducted into the corresponding control group. Twenty-four fish were randomly placed in each container, and exposed for 60 h to derive the death time of each fish with different TDG saturation levels. All experiments were conducted in duplicate under the same condition.

2.3.3 Avoidance response test

A two-channel, open wooden box was constructed (Fig. 3), so that when the experimental fish distinguish the supersaturated water and the equilibrated water, they could choose to avoid entering into supersaturated water or not. Each channel is 0.225 m wide, 0.980 m long, and 0.120 m high. Two inlets were provided, one inlet with supersaturated water and the other with equilibrated water. Fourteen holes with a diameter of 0.01 m for outlet at the bottom of the box were added. Different levels of supersaturated water (105%, 115%, 125%, 135%, and 145%) flowed through one channel, while the equilibrated water (99%) flowed through the other one. The depth of both supersaturated water and equilibrated water was kept at 0.08 m by controlling the balance of inflow and outflow at 0.05 L/s.

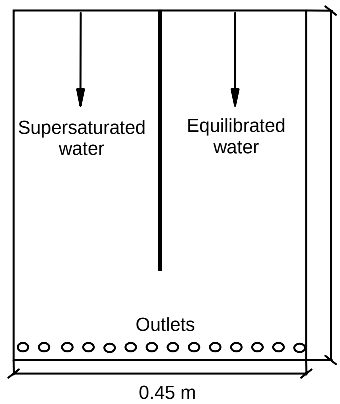

Fig. 3 Top view of choice box used in experiments
The arrows indicate the direction of water flow from each treatment channel

Initially, 18 to 22 fish were randomly placed into the water with different gas supersaturation levels (105%, 115%, 125%, 135%, and 145%) through supersaturated water channel. Then every fish's position was recorded after 5 min. The experimental circumstance was the same as the water for acclimation, and the supersaturated water could be produced by the system (Fig. 1). All experiments were conducted under natural light during the day, and the behavior of the fish was recorded by video. All experiments were conducted in three sessions under the same condition, and the experimental fish were not reused (as the fish may learn and have latent stress). The gas supersaturation was monitored at the beginning and at the end of each replication.

2.4 Statistical analysis

Statistical analysis was conducted by SPSS software Version 13.0. Median lethal time (LT_{50}) was used to assess the lethal responses in the juvenile rock carp in different levels of supersaturated water, and avoidance percentage (AP) was used to determine the avoidance ability of the experimental fish: AP=$(X-Y)/N\times100\%$, where X is the number of fish in equilibrated water, Y is the number of fish in supersaturated water, and N is equal to $X+Y$ (Qiu, 1992).

3 Results

3.1 Acute lethality

The LT_{50} of the juvenile rock carp in different levels of gas supersaturation is shown in Fig. 4. The juveniles died within a few hours after being put into the supersaturated water with levels of 130%, 135%, 140%, and 145%. However, the LT_{50} dramatically reduces when the gas saturation level is 125%. The mortality is minimal when the fish are in the supersaturation water with levels of 105% and 115% for 60 h, so the LT_{50} in these cases could be neglected.

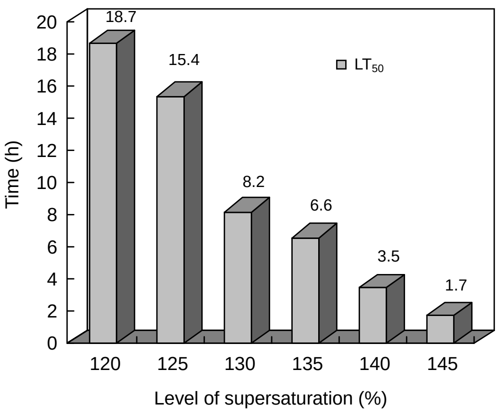

Fig. 4 Different LT_{50} under different levels of supersaturated water

3.2 Avoidance responses

The juvenile rock carp exhibited strong avoidance responses in the supersaturated water. The avoidance percentages are listed in Table 1. The data show that the avoidance percentage is larger than 90% when the gas supersaturation is above 135%, and the avoidance percentage decreases as the gas supersaturation reduces. In addition, the experiments show that the fish exhibit an obvious preference for supersaturated water when the gas supersaturation is below 115%.

Table 1 Two-choice trials for juvenile rock carp

TDG (%)	Fish number of every trial			Mean avoidance percentage (%)
	No. 1	No. 2	No. 3	
145	18	18	20	100.0
135	21	20	18	91.0
125	18	20	21	63.0
115	22	19	18	15.0
105	18	22	19	8.5

The avoidance percentages in the water with different levels of gas supersaturation (105%, 115%, 125%, 135%, and 145%) were analyzed by *F*-test of analysis of variance (ANOVA). The results show that there is statically significant difference in avoidance percentages at different levels of supersaturation ($F=599.92>F_{0.01(3,10)}=6.55$, $P<0.01$).

4 Discussion

4.1 Fish species and sensitive life stage

Different susceptibilities to gas supersaturation depend on the fish species. Alec *et al.* (1995) reported the LT_{50} for juvenile salmonids is 60 h at 120% TDG saturation and 6 h at 130% saturation, when the water is 28 cm deep. Beeman *et al.* (2003) found the LT_{50} for juvenile northern pike minnow is 15.3 h at 125% saturation and 10 h at 130% saturation. Some fish, such as rainbow, chinook, black bullhead, and northern pike minnow may detect and avoid moderate to high levels of supersaturation, while others seem to lack this ability (Stevens *et al.*, 1980; Weitkamp and Katz, 1980; Lund and Heggberget, 1985; Beeman and Maule, 2006). The present study indicates that juvenile rock carp may detect supersaturated water and move laterally away from it.

It has been documented that the tolerance to supersaturation may arise as fish develop (Weitkamp and Katz, 1980; Mesa and Warren, 1997). Fidler and Miller (1997) concluded that smaller juvenile salmonids are most sensitive to elevated TDG levels. The exclusive use of juvenile rock carp in the trials is valuable because the life cycle stage is believed to be most affected by gas supersaturation. After understanding the effects of gas supersaturation on the minimal resistant life stage, the worst case scenario in this kind of fish is understood.

4.2 Temperature and water level variability

The gas solubility in water is inversely proportional to the temperature. Under one-atmosphere of pressure, 1 °C increase in temperature will result in 2% increase in the TDG saturation (Nebeker *et al*., 1978; Schneider, 2003; Arntzen *et al*., 2009). Many researchers have found that fish in supersaturated water with water depth compensation (increasing the water depth) have a lower mortality than fish in the water with the same supersaturation level, but without water depth compensation (Ebel, 1969; Meekin and Turner, 1974; Blahm *et al*., 1976; Weitkamp, 1976; Heccbercet, 1984). Water with a saturation level of 110% tends to lose excess air when it is at surface pressure. However, at the hydrostatic pressure of 1 m deep water, the air does not tend to come out of solution (Knittel *et al*., 1980). The fish have a greater tolerance under the condition which allows them to reach deeper levels (due to hydrostatic pressure compensation). For example, at 120% of saturation relative to surface pressure, a fish at a depth of 2 m only experiences 100% of saturation. Thus, the experiments were carried out under constant water temperature of 25 °C and water level of 0.2 m in order to keep the TDG saturation stable and minimize depth compensation.

4.3 Safety of gas supersaturation

The low mortality of the fish in the water with a supersaturation level of 115% for 60 h indicated that there is no acute lethality in juvenile rock carp when they are in water with the gas saturation below 115% under the temperature of 25 °C and with a water level of 0.2 m. Further, they exhibit a preference to supersaturated water when the gas supersaturation is below 115%. This experiment indicated that the juveniles can survive in water with a level of 115% saturation, possibly because the concentration causing avoidance behavior is often lower than the lethal concentration. If all the experimental fish show avoidance behaviors at a certain concentration, however, this concentration is equivalent to the lethal concentration (Lutz, 1995). The results are in accordance with the report written by NOAA, which suggests that TDG supersaturation levels between 110% and 120% had minimal impact on aquatic biota in river environments (NOAA, 1995).

4.4 TDG control

Investigation of GBD showed that fish can recover from sublethal effects and signs of GBD, if they are removed from the supersaturated water or given sufficient hydrostatic compensation. Knittle *et al*. (1980) had placed juvenile steelhead at a depth of 3 m for 3 h in order to let the fish fully recover from near-lethal surface exposures to 130% TDG. Hans *et al*. (1999) reported bubbles in gill filaments of spring chinook salmon were almost completely dissipated within 2 h after the fish were transferred to normal water. The different LT_{50} in the experiments shows the lethal extent of different levels of TDG supersaturations, and can determine the time over which the juveniles can recover from sublethal levels. The gas supersaturation criteria could be further set down by additional investigation on other species and field observations.

5 Conclusions

The results show that 120% supersaturation is the threshold of acute lethal levels, and 115% saturation is safe for the juvenile rock carp. It can provide an initial evaluation for the effects of TDG saturation on domestic species in the Yangtze River. A gas supersaturation criterion could be set to protect the downstream aquatic environment of the Yangtze River.

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