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# The Effect of Acute Stress on Post-Stress Oxygen Consumption Rate in Southern Catfish, *Silurus meridionalis* Chen

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**Abstract:** The post-stress oxygen consumption rate ($VO_2$) was investigated in southern catfish (47.43±3.92 g) after different acute stress. The stress treatment were chasing for 2.5 min (CH), air exposure for 2.5 min (AE), 12.5°C coldwater bath for 2.5 min (CB), 2.5 min chasing plus 2.5 min air exposure (CA) and chasing at 12.5°C for 2.5 min (CC), respectively. All water and air temperature was 25°C except that of coldwater bath group. $VO_2$ of all groups were increased immediately after stress (coldwater bath was increased a little slower) and slowly return to a pre-stress level. The $VO_{2peak}$ of CC group was significantly higher than those of all other groups ($p<0.05$). The $VO_{2peak}$ of CH group was significantly higher than that of CE group, while the latter was significantly higher than those of AE and CB groups ($p<0.05$). The $VO_{2peak}/VO_{2rest}$ in both chasing groups (CH and CC) were significantly higher than those of other groups ($p<0.05$). The excess post-stress oxygen consumption rates (EPOC) of both chase groups were significantly larger that of CE group ($p<0.05$), while the latter was significantly larger than those of CB and AE groups ($p<0.05$). It suggested that: the $VO_2$ response of southern catfish to chase was larger that air exposure and acute low-temperature stress, while air exposure and low-temperature stress might have little effects on $VO_2$ response; Compared to chase effect, added air exposure treatment after chase lowered the post-stress $VO_2$ response, while coldwater bath chase might elevated the post-stress $VO_2$ response and as a sit-and-wait forager with poor aerobic and anaerobic capacity, the stress response of southern catfish was relatively lower.

**Key words:** Acute stress, *Silurus meridionalis* Chen, oxygen consumption rate ($VO_2$), Excess Post-stress Oxygen Consumption (EPOC)

## INTRODUCTION

In fish culture, stress associated with the numerous naturally occurring changes in chemical, biological and physical disturbances in the aquatic environment results in adverse growth, poor food utilization, increased susceptibility to disease and even death (Davies and Schreck, 1997; Noga *et al*., 1998; Kubilay, 2002; Ruyet *et al*., 2003; Dautremepuits *et al*., 2004). Fish under aquaculture conditions are often subject to stressors, such as handling, crowding, transporting and changing water qnality that elicit changes in the animal's physiological state, which is interpreted as the stress response (Ruyet *et al*., 2003; Dautremepuits *et al*., 2004). The responses of fish to stressors were extensively studied in the past several decades (Ruyet *et al*., 2003). Most of such study had focused on the physiological response to chronic stressor such as stock density (Mazur and Iwama, 1993), dissolved oxygen content (Ruyet *et al*., 2003) and noxious chemicals

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(Dautremepuits *et al*., 2004). With little effort on stress-related response of fish to acute stressors such as chase, air exposure and cold stress etc, all might be daily physiological problem in fishery industry (Kubilay, 2002).

The evolutionary and life history background of a given fish differed a lot and the response to acute stress of different fish species might also be different. Such investigation might be help to understand the adaptation and evolution of organism to acute stress. Southern catfish (*Silurus meridionalis* Chen) is a local warmwater sit-and-wait fish species. Several energetics studies of this species were documented and such research showed that it has particular physiological trait such as low maintain energy requirement, poor swimming ability, higher postprandial metabolic scope etc. (Fu and Xie, 2004; Fu *et al*., 2005a, b, c). The effect of acute stress on southern catfish and its comparison to other kind of fish species might be an interesting issue. Further more, southern catfish was one of the most extensive culture catfish in china. The investigation on stress response in southern catfish might also provide important data for fishery industry. So the post-stress $VO_2$ response to chase, air exposure and coldwater bath were investigated using southern catfish as experimental model. The aims of this study were to: (1) document useful data for stress physiology and fishery industry. (2) investigate and compare $VO_2$ response to different kind stress in southern catfish. (3) investigate the combined response of southern catfish under two kinds of stress.

## MATERIALS AND METHODS

### Experiment Animals

Juvenile fish were obtained from a local live market and acclimated in a rearing system 4 weeks prior to the experiment. Temperature of dechlorinated freshwater was maintained at 25.0±1.0°C, oxygen content was kept above 7 ppm. During this period the fish were fed to satiate once every 3 days. A 14L: 10D photoperiod was used to simulate natural light cycle.

### Respirometer and Measurement of $VO_2$

$VO_2$ for individual fish was measured by using a continuous flow respirometer (chamber volume: 0.12 L) modified from the design of Fu *et al*. (2005a). Fish were held in any given experiment chamber and one chamber without a fish acted as a control for background $O_2$ consumption. The following formula was used to calculate oxygen consumption of individual fish ($VO_{2\text{per fish}}$, mg $O_2$ $h^{-1}$) of individual fish:

$$VO_{2\text{per fish}} = \Delta O_2 \times v \quad (1)$$

where $\Delta O_2$ is the difference (mg $O_2 L^{-1}$) in oxygen concentration between an experimental chamber and the control chamber, v is the velocity of water flow in a chamber (L $h^{-1}$). $VO_{2\text{per fish}}$ was adjusted to a standard body mass of 1kg using a mass exponent of 0.75 (Fu *et al*., 2006). Standardized value of metabolic rate was calculated by the following formula:

$$VO_2 = (1/m)^{0.75} VO_{2\text{per fish}} \quad (2)$$

where $VO_2$ is the standardized oxygen consumption rate and m is the body mass of the fish (kg).

Dissolved oxygen concentration was measured at the outlet of the chamber by an oxymeter (HQ20, Hach Company, Loveland, Colorado, USA). The flow rate of water through the respirometer chamber was measured by collecting the water outflow from each tube into a beaker over 1 min (Fu *et al*., 2005a).

**Experiment Protocol**

After acclimation, fish with similar weight (47.43±3.92 g) were selected and transferred to respirometer chamber after 2 d fast. The $VO_2$ was measured after another day's fast and regarded as $VO_{2rest}$. For chase group (CH), fish were chased with a hand-net for 2.5 min in a circle trough (all fish were exhaustive after chase, the process was fully described Fu *et al*. (2006), then placed into respirometer chambers immediately. The post-exercise $VO_2$ were measured at 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35 and 40 min after they were transferred to chambers. For air exposure group (AE), after the measure of $VO_{2rest}$, the chamber was released from respirometer and gently elevated from water bath. After 2.5 min air exposure (air temperature was maintained at 25.0±1.0°C) the chamber was re-located and $VO_2$ was record as that of CH group. For Coldwater Bath group (CB), fish was transferred to cold water (about 20 L) at 12.5±0.5°C for 2.5 minand then transferred to chambers for measure of $VO_2$. For chase and air exposure group (CA), fish was chased as described in CH group and then air exposed for another 2.5 min before transferred to chamber. For chase and coldwater bath group (CC), fish was chase in 12.5±0.5°C water for 2.5 minand then placed into respirometer chambers immediately for measure of $VO_2$.

During metabolic recovery process, the flow rate of chambers was about 0.5 L $min^{-1}$ and the exchange of water was more than 99% at 2 min with the 0.12-L chamber (Steffensen, 1989).

**Experiment Parameters and Data Analysis**

Five parameters were used to evaluate stress $VO_2$ response: (1) resting oxygen consumption rate ($VO_{2rest}$, mg $O_2h^{-1}$): the $VO_2$ of southern catfish before stress (fast for 3 d). (2) peak oxygen consumption rate ($VO_{2peak}$, mg $O_2$ $h^{-1}$): the maximal $VO_2$ during recovery process. (3) factorial scope: the $VO_{2peak}$ was divided by $VO_{2rest}$. (4) duration: the time course from start to $VO_2$ was not significantly different from pre-stress level. (5) Excess post-stress oxygen consumption (EPOC, mg $O_2$): the oxygen consumption that exceeds $VO_{2rest}$ during recovery process.

The effects of stress on $VO_2$ were compared using a one-way ANOVA and least significant difference (LSD) test. A p-value lowers than 0.05 was considered statistically significant and all data were presented as means±1SEM. The STATISTICA 4.5 (StatSoft Inc) was used for data analysis.

## RESULTS

Neither body mass nor $VO_{2rest}$ differed significantly before experiment (Table 1). After stress, $VO_2$ of fish in all treatment groups increased immediately and slowly returned to pre-stress level at about 20 to 35 min after stress (Fig. 1). The $VO_2$ curve exhibited profound difference among different treatments. The durations of stress $VO_2$ response were 25, 15, 20, 25 and 30 min for CH, AE, CB, CA and CC groups, respectively. The $VO_{2peak}$ of CC group was significantly larger than those of all other groups (p<0.05). The $VO_{2peak}$ of CH group was significantly larger that of CA group (p<0.05), while the latter was significantly larger than those of AE and CB groups (p<0.05). The $VO_{2peak}/VO_{2rest}$ of CH

Table 1: The effect of stress on $VO_2$ in southern catfish (Means±SE, N = 5)

| Data | Chase (CH) | Air exposure (AE) | Coldwater bath (CB) | Chase and air exposure (CA) | Chase and cold bath (CC) |
|---|---|---|---|---|---|
| Body mass (g) | 39.14±3.91 | 56.55±6.95 | 43.62±3.18 | 50.36±1.45 | 47.46±4.13 |
| $VO_{2rest}$, mg $O_2$ $h^{-1}$ | 52.61±2.30 | 47.31±3.36 | 67.00±8.69 | 67.47±5.19 | 64.58±4.49 |
| $VO_{2peak}$, mg $O_2$ $h^{-1}$ | 204.30±4.80$^b$ | 139.28±7.46$^d$ | 143.59±10.99$^d$ | 169.43±7.46$^c$ | 225.39±7.23$^a$ |
| $VO_{2peak}/VO_{2rest}$ | 3.92±0.26$^a$ | 2.90±0.21$^b$ | 2.50±0.31$^b$ | 2.65±0.18$^b$ | 3.73±0.42$^a$ |
| Duration, min | 25 | 15 | 20 | 25 | 30 |
| EPOC, mg$O_2$ | 35.22±2.44$^{ab}$ | 10.41±3.34$^c$ | 11.46±7.93$^c$ | 29.00±1.12$^b$ | 40.83±4.11$^a$ |

[1:] Values in each row without a common superscript are significantly different (p<0.05)

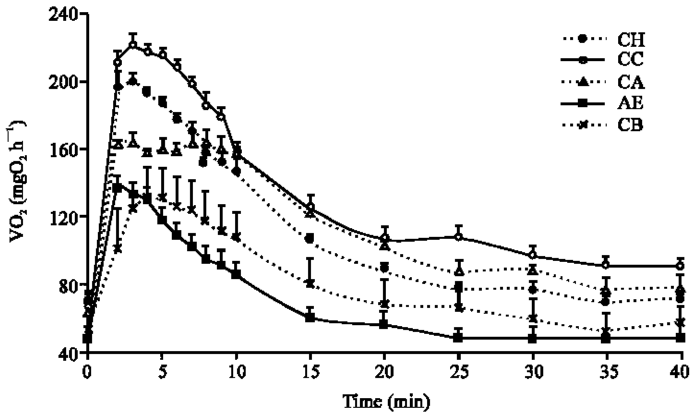


Fig. 1: The effect of stress on $VO_2$ in southern catfish (Means±SE, N = 5). (CH: chase for 2.5 min, AE: air exposure for 2.5 min, CB: coldwater bath for 2.5 min, CA: air exposed for 2.5 min after 2.5 min chase, CC: chase for 2.5 min in 12.5±0.5°C water)

and CC groups were significantly larger than those of all other groups ($p<0.05$). The EPOC of CH and CA groups was significantly larger than that of CA group ($p<0.05$), while the latter was significantly larger than those of AE and CB groups ($p<0.05$).

## DISCUSSION

The $VO_{2rest}$ in this study ranged from 47.31 to 67.47 mg $O_2$ $h^{-1}$. It was similar to previous study on this species (Fu and Xie, 2004; Fu *et al*., 2005). The $VO_{2peak}$ of chase group was about 200 mg $O_2$ $h^{-1}$ adjusted to 1 kg body mass. They were also similar to previous work in southern catfish under similar condition (Fu *et al*., 2006a). But the $VO_{2rest}$ and $VO_{2peak}$ were lower than most of documented values in other fish species (Fu *et al*., 2006b; Lee *et al*., 2003). It might relate to its ecological habit. As a large sit-and-wait forager, southern catfish was on the top-level of food chain (Fu *et al*., 2005b) and there was little risk of predation in nature. The aerobic and anaerobic capacity and hence locomotion performance were fairly poor as suggested in previous work and this study (Fu *et al*., 2005b). The benefit of such energetics strategy was lower maintaining energy expenditure and hence higher energy utilization (more than 60%, one of the highest energy efficiency in fish species).

In energetics research about stress response in fish species, $VO_{2peak}$ was often used as an indictor of stress intensity (Radull *et al*., 2002). Along with $VO_{2peak}$, duration of response and total exceed energy expenditure during recovery process (EPOC) might be more suitable for valuing the magnitude of stress. The phosphagen replenishment in skeletal muscle and resaturation of hemoglobin and myoglobin stores in muscle and blood are thought to be major contributors to EPOC in the early minutes post-chase (Bahr, 1992). It might be the same situation in the early minutes post air exposure and post coldwater bath. The physiological explanations for prolonged period (several minutes to the end of recovery process) of elevated metabolism might be the high levels of circulating catecholamines and hence elevated cardiac and ventilatory work, clearance of accumulated lactate, restoration of depleted glycogen stores, ion redistribution, tissue repair, mitochondrial uncoupling, pH depression and substrate cycling within both the gluconeogenic-glycolytic and triglyceride-fatty acid cycles as suggested by chase stress (Gleeson and Hancock, 2002). The $VO_{2peak}$ in fish after stress may be decided by two factors. First, if the stress is strong enough, the $VO_{2peak}$ might be limited by aerobic capacity of a given species. If not, the $VO_{2peak}$ may be decided by the rapid component of

EPOC list above. So $VO_{2peak}$ may be a suitable indictor of early minute stress response. But the important physiological process which buildup the prolong stress response component may not well related with $VO_{2peak}$. However, such component may well estimated by duration and magnitude of total stress response (EPOC). In this study, the EPOC of chase was also much lower than those of previous work (Lee *et al*., 2003), which again suggested the chase stress was much lower than other fish species.

Although the chase elicited $VO_2$ response was much lower than most of other fish species, the $VO_2$ response to chase stress was still the largest among all stress treatments. Similar result was documented before in spotted grunter (*Pomadasys commersonnii*) (Radull *et al*., 2002). It perhaps due to the chase stress was more like prey (or preyer)-encounter situation. Under the selection press of enemy avoiding and success feeding, organism might mobilize all potential.

Compared to chase, only about 70% $VO_{2peak}$ and 30% EPOC were elicited by air exposure. The 2.5 min air exposure might have little impairment on southern catfish for its air-respiration capacity. Pilot experiment found the operation process of air exposure could elicit about 10 $mgO_2$ EPOC. It meant that the EPOC of air exposure was mainly elicited by operation stress rather than air exposure stress. We have ever measure the EPOC of southern catfish under 10 to 20 min air exposure. We found when exposure to 5 to 10 min, southern catfish showed some movement, which elicited higher $VO_{2peak}$ and EPOC. Even under such condition, the maximal EPOC was still not more than 20 mg $O_2$. It suggested that the physiology response to air exposure was much smaller than that of chase. Some research found the structure of branchia might be impaired under air exposure (Chandra and Banerjee, 2003) and $NH_4^+$ might be accumulated during air exposure. Such change may detrimental to respiratory function in fish species (Polez *et al*., 2003). We found all experimental fish feed normally 12 h after treatment, so we think the experiment might not had profound detriment on southern catfish in this study.

The $VO_2$ response to acute cold stress was similar to that of air exposure. The $VO_2$ at 2 min after cold stress was much lower than those of other groups. It suggested southern catfish was more like a conformer rather regulator as to its adaptation to acute cold enviroument. It was consisted to its natural bahaviour. As a warmwater fish species, the $VO_{2rest}$ was profound decreased in winter. Fish usually stop feeding in winter when ambient temperature drop below 12 to 14°C and exhibit hibernant symptom (extremely lower ventilation frequency, inaction and coma etc) when ambient temperature drop below 10°C according to our laboratory observation. But another warmwater small catfish-darkbarbel catfish (*Pelteobag vachelli* Richardson) showed totally different response to acute temperature stress and long temperature acclimation compared to southern catfish (Fu *et al*., 2006a). The $VO_{2peak}$ response to acute cold stress might even higher than that of chase in darkbarbel catfish (Fu *et al*., 2006a). Darkbarbel catfish do not stop feeding at winter and $VO_2$ decreased much less than that of southern catfish (the $VO_2$ of dark barbell catfish was smaller than that of southern catfish in summer but higher than that of southern catfish in winter). Such physiology difference related to temperature stress between two fish species located in same water area might be an interesting issue in future.

In natural, fish may cope with more than one stress. Whether the $VO_2$ response would add or to what extend would two different stresses add was one aim of this study. In this study, compared to chase treatment, both $VO_{2peak}$ and EPOC decreased. It suggested that 2.5 min air exposure have little impact on physiological function in southern catfish. The decrease of $VO_{2peak}$ and EPOC might be results of air respiration during 2.5 min air exposure. On the contrary, the stress response of chase and acute cold may add from this study since $VO_{2peak}$ and EPOC of CC group was higher than those of both chase and cold stress group.

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