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Physiological and Biochemical Responses of Bighead Carp (*Aristichthys Nobilis*) Due To the Effects of Acute High-Temperature Stress

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Abstract: **This study was undertaken to investigate the effects of acute high-temperature stress on physiological and biochemical responses of Bighead carp (*Aristichthys nobilis*). For this purpose, we select similar size of fish with a mean weight of 250 ± 66 g and randomly were assigned into 6 tanks (300 liter/tank) with three replicates at a stocking density of 9 fish per tank, fed with a commercial diet. Fish were reared at 28 °C for 10-weeks, then first sample was collected after 24 hr thereafter a subsample of 36 fish from the two groups were exposed gradually every 3h to 38 °C for 24 hr and samples of blood collected at each step. The results showed that the effect of acute high-temperatures lead to stress the fish thereby affected the physiological and biochemical responses. Increased temperature leads to decrease the levels of plasma cholesterol and triglyceride while alanine aminotransferase, alkalin phosphatase and glucose activities were increased significantly (*P<0.05*) under acute high-temperature stress. The overall results of the present studies indicate that Bighead carp are highly susceptible to changes in environmental temperature and rapid intervention is required in response to temperature disruptions during aquaculture. This study expected to help aquaculturists monitor tolerable temperature and time range of exposure to stress. Aquaculturists will have to be on guard regulating toxic elements in fish culture emanating from feed additives, pollutants and hormones.**

Keywords: **Bighead carp (*Aristichthys nobilis*), biochemical parameters, stress response, temperature.**

I. INTRODUCTION

Fish especially in controlled environments are constantly exposed to acute or chronic stressors such as changes in ambient temperature, transport and handling stress, high stocking densities and poor water quality (Ashley, 2007; Xu et al., 2011; Liu et al., 2012). The body temperature of most fishes equilibrates rapidly with ambient temperature, so water temperature is suggested to be abiotic master factor which virtually controls and limits all biochemical, physiological and life history activities (Beyers & Rice, 2002; Donaldson et al., 2008). Furthermore, temperatures beyond tolerable ranges of above 35 °C cause acute or chronic stress. Acute temperature increases trigger primary and secondary stress responses, which produces osmoregulatory and metabolic effects in many fish species (Ackerman et al., 2000; Basu et al., 2001; Gollock, 2002). It may also increase susceptibility to infection and even death when temperatures increase above 38 °C (Bermudes et al., 2010).

Physiological responses of fish to stressors have been broadly categorized into primary, secondary and tertiary responses. Primary responses involves the initial neuroendocrine response that includes the release of catecholamines from chromaffin tissues, and the stimulation of the hypothalamic-pituitary-interrenal (HPI) axis culminating to the release of stress hormones, cathecolamines and cortisol circulation (Randall and Perry, 1992; Reid et al., 1998; Mommsen et al., 1999). Secondary response comprises a wide range of changes caused to a large extent by stress hormones in blood, organs and tissues of the animal. They are all related to physiological adjustments for instance in metabolism, respiration, acid-base status, immune function and cellular responses (Iwama et al., 1998; Mommsen et al., 1999). Additionally, tertiary responses in fish refer changes in whole-animal performance such as changes in health condition, growth, overall resistance to disease, metabolic scope for activity, behaviour and ultimately survival (Wedemeyer et al., 1990). This grouping is simplistic, however, as stress, depending on its magnitude and duration may affect fish at all levels from molecular and biochemical to population and community (Adams, 1990). Although, many studies have addressed the effects of acute and chronic high-temperature stress in different fish species (Bagnyukova et al., 2007; HoweCasanova et al., 2008; Desai and Singh, 2009; Tropea et al., 2010; Glencross and Bermudes, 2011; Ming et al., 2012; Cheng et al., 2015; Liu et al., 2016), but there has been no studies on Bighead carp (*Aristichthys nobilis*).

Bighead carp is one of the most economically important freshwater filter-feeding fish in Asia. It constitutes the majority of carp aquaculture production in China yielding an annual production of 3,253,143 tons as of 2014 (FAO, Fish stat, 2014). Bighead carp can tolerate extreme changes in water temperature ranging from those normally found in cold, temperate as well as those in tropical climates. The maximum temperature in which Bighead carp can survive is 38°C (USGS, 2005). The lowest temperature they can tolerate is close to freezing at 1°C (ISSG, 2005). However, their preferred temperature for optimal reproduction is about 25°C (USGS, 2005). Bighead carp feeds on both phytoplankton and zooplankton, and phytoplankton constitutes a substantial part of their diet (Opuszynski, 1981). Because of their filter-feeding habits, Bighead can be used as a means to control algal blooms (Cremer and Smitherman, 1980). As such, it is a highly desirable aquacultural commodity, and is now widely exported from China to more than 70 other countries.

In the present study we investigated the effects of acute high-temperature stress on physiological and biochemical responses of Bighead carp (*A. nobilis*).

II. MATERIALS AND METHODS

Experimental system and fish rearing:

Healthy Bighead carp (*A. nobilis*) were obtained from Nanquan fish farm, which is part of the Freshwater Fisheries Research Center, Chinese Academy of Fishery Science in Wuxi, China. Experiments were carried out in a closed freshwater recirculating system with a water flow rate of about 3 L/min and continuous aeration. 54 fish were acclimatized in 300 L dechlorinated freshwater tanks for two weeks, and fed to near satiation three times daily with a commercial diet. After this adaptation period, fish with a mean weight of 250 ± 66 g were selected and randomly assigned into 6 tanks with three replicates at a stocking density of 9 fish per tank. Uneaten feed and feces were cleared on a daily basis with a siphon. During the experiment, the water quality parameters maintained were: temperature, 28 °C; dissolved oxygen concentration, > 6.0 mg/L; total ammonia-nitrogen concentration, < 0.05 mg/L; pH, 7.0 – 7.5. These conditions were monitored weekly. The photoperiod was 12 hr light/12 hr dark. All husbandry procedures and experiments were carried out in accordance with local and international animal welfare policies stipulated by scientific research protocols (CAFS) and the Ministry of Agriculture, PR China (FAO, 2004).

Temperature challenge experiments:

Prior to the challenge experiment, fish were reared at 28 °C for 10 weeks. A first sample was collected after 24 hr thereafter a subsample of 36 fish from the two groups were exposed gradually every 3hr to 38 °C for 24 hr and samples were collected at each step. A 12/12 hr light/dark, as well as pH, 7.0-7.5; ammonia-nitrogen concentration, < 0.05 mg/L; dissolved oxygen > 6.0 mg/L were constant during all the duration of the experiment. Human interference was minimized to prevent additional stress to the fish.

Sample collection:

At the end of the 10 week experiment, sampling before stress was conducted 24 hr after the last feeding of fish in order to evacuate the contents of the alimentary tract. Blood samples were collected once before stress (0 hr) and at (3, 6, 12, and

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24 hr) during acute high-temperature stress, respectively. Fish were quickly anesthetized with MS-222 (Tricaine methanesulfonate, Sigma, USA) in the concentration of 100 mg/L to minimize stress before collecting blood, then blood was sampled immediately from the caudal vein using 5-mL medical syringe. The blood was held for 2 hr in a refrigerator at 4°C, and then subsequently centrifuged for 10 min at 4°C and 3500 rpm to obtain the plasma. The supernatant was removed and stored at −80°C until further use. Fish were sacrificed immediately by spinal transaction after blood collection and the liver were removed and immediately frozen in liquid nitrogen and kept at −80°C for enzymatic activities.

Plasma biochemical Parameters:

Alanine aminotransferase (ALT) activities were determined by a colorimetric test kit (Mindray Bio Medical Co., Ltd., Shenzhen, PR China) according to Reitman and Frankel, (1957). Total cholesterol (TC) and Triacylglycerol (TG) activities were determined by the colorimetric test kit (Mindray Bio Medical Co., Ltd., Shenzhen, PR China) according to the method described in Habte-Tsion et al. (2015b). The levels of glucose content (GLU) and alkaline phosphates (ALP) were determined by colorimetric test kits (Mindray Bio Medical Co., Ltd., Shenzhen, PR China) according to the protocols provided by the manufacturer of using an auto biochemical analyzer (BS-400, Mindray, Shenzhen, PR China). All kits were specially designed for fish detection.

Statistical analysis:

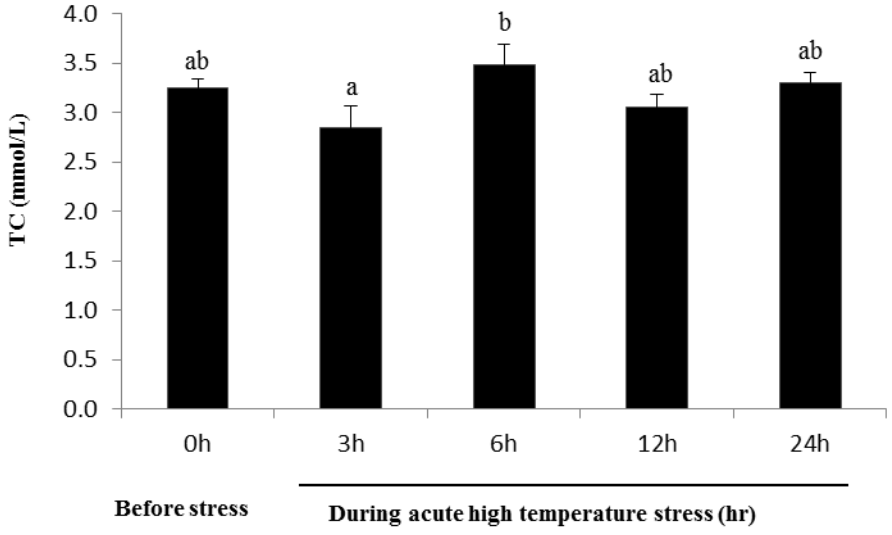
All results were statistically evaluated by using a statistical package for social sciences (SPSS) program for windows (version 19.0, Chicago, IL, USA). Data were analysed by a one-way ANOVA followed by Duncan's multiple range tests (DMRT). *P* value ≤ 0.05 was considered to be statistically significant. Data for each parameter were expressed as mean ± standard deviation in three replicate.

III. RESULTS

The effect of acute high-temperature stress on plasma biochemical composition:

The effects of acute high-temperature stress on plasma total cholesterol (TC) and Triacylglycerol (TG) of Bighead carp (*A. nobilis*) are shown in (Fig. 1). The effects of acute high-temperature stress on plasma TC activity are illustrated in (Fig. 1A). Plasma TC activity decreased during acute high-temperature stress at 3hr ($P < 0.05$) compared to the control group. It then increased and reached a maximum value at 6 hr before decreasing again (Fig. 1A). No significant differences were observed between control and treatment group except for 3 and 6hr group.

The effects of acute high-temperature stress on plasma TG activity were shown in (Fig. 1B). Plasma TG activity significantly decreased during acute high-temperature stress at 3hr ($P < 0.05$) compared to the control group. No significant differences were observed between control and 6 hr treatment group.



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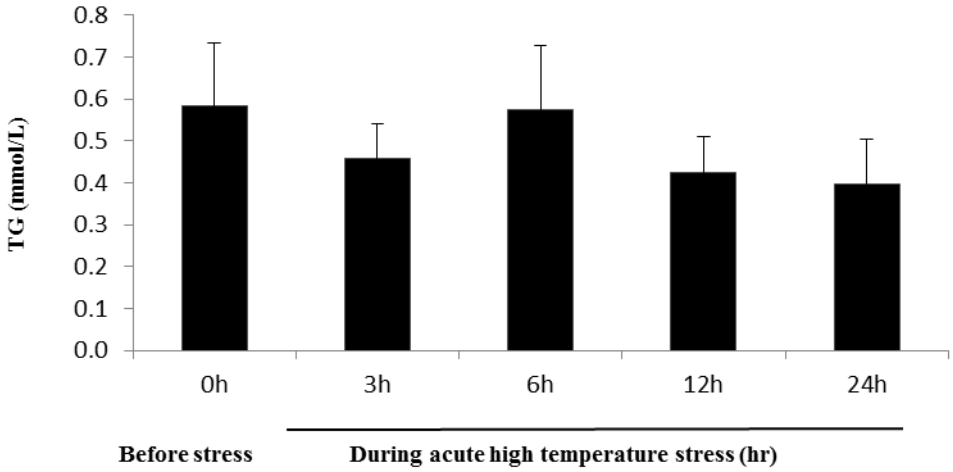


B

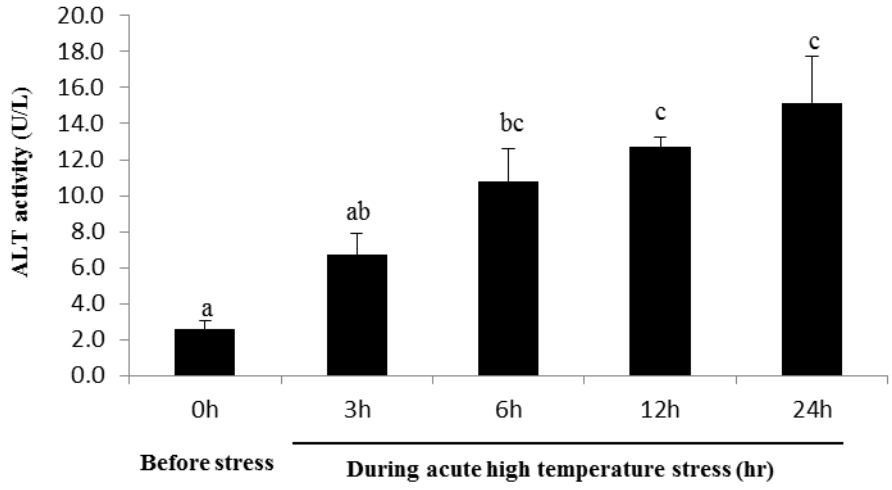
Fig.1. Effect of acute high-temperature stress on plasma TC (A) and TG (B) in Bighead carp (*Aristichthys nobilis*). *Note* Data are expressed as mean ± SE (n=9). Significant differences ($P < 0.05$) in each sampling time point were calculated using Duncan's multiple range

The effect of acute high-temperature stress on plasma stress response:

The effects of acute high-temperature stress on plasma alanine aminotransferase (ALT), alkaline phosphates (ALP) and glucose content (GLU) of Bighead carp (*A. nobilis*) are shown in (Fig. 2). The effects of acute high-temperature stress on plasma ALT activity are illustrated in (Fig. 2A). Compared to the control the plasma ALT activity significantly increased from 3hr and reached a maximum at 24hr ($P < 0.05$). The highest level of plasma ALT activity were observed after 24 hrs ($P < 0.05$) during acute high-temperature stress and never returned to pre-treatment levels compared with levels before even in the control group (Fig. 2A).

Temporal changes in plasma ALP level during exposure to acute high-temperature stress are illustrated in (Fig. 2B). Plasma ALP activity decreased at 3hr and shows no significantly different compared to the control group. It then increased and reached a maximum value at 24 hr during acute high-temperature stress (Fig. 2B).

The effects of acute high-temperature stress on plasma GLU activity were shown in (Fig. 2C). Compared to the control, plasma GLU activity was increased significantly and reached the highest level at 3 hrs ($P < 0.05$), thereafter tended to decrease when fish were under acute high-temperature stress (Fig. 2C).



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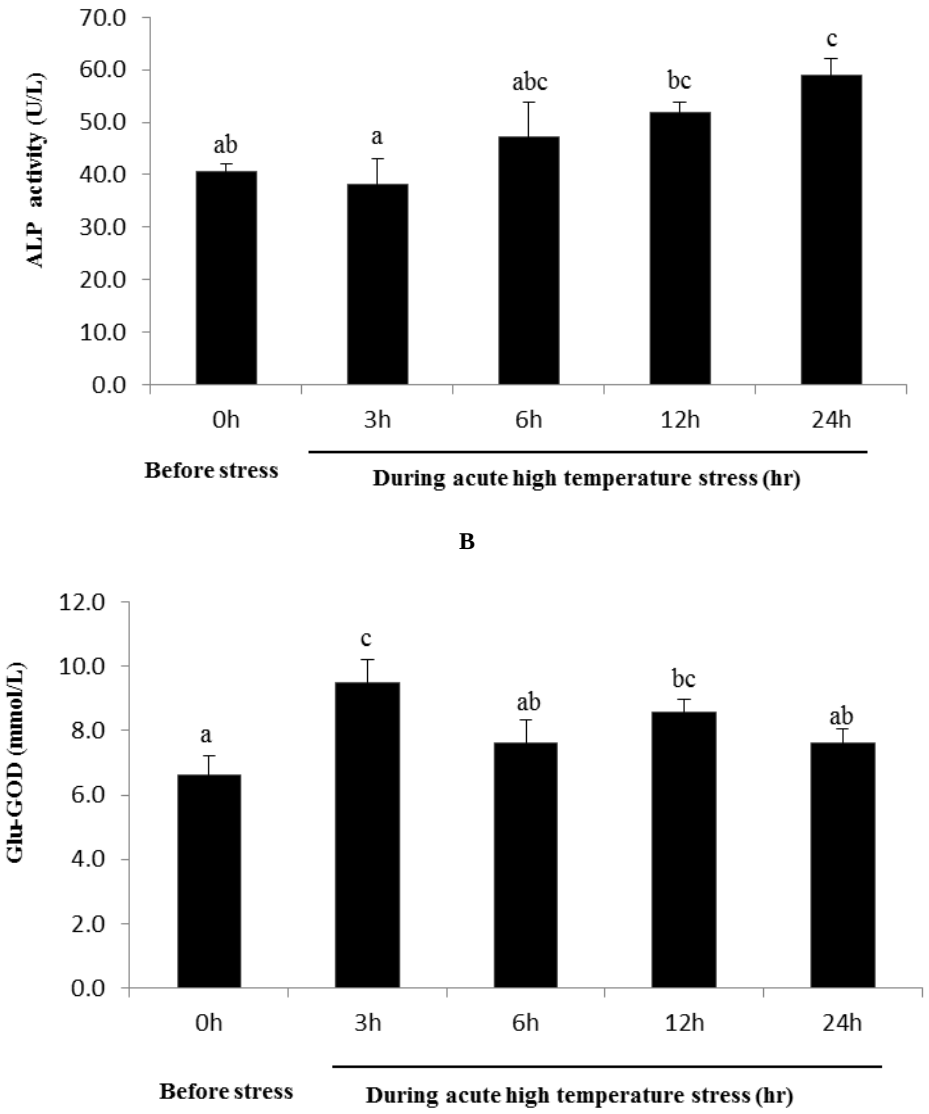

Fig.2. Effect of acute high-temperature stress on plasma ALT (A), ALP (B) and GLU (C) in Bighead carp (*Aristichthys nobilis*). *Note* Data are expressed as mean ± SE (n=9). Significant differences ($P < 0.05$) in each sampling time point were calculated using Duncan's multiple range

IV. DISCUSSION

Temperature is a major determinant of cellular physiology for ectotherms, as it affects physiological processes by a direct effect on the rates of biochemical reactions. When the temperature reaches the upper extreme of the tolerated range it can cause stress and this stress can either be acute or chronic. The response of fish to acute or chronic stress is reflected as changes in some enzyme activities especially key enzymes of biotransformation systems of organisms which can be used as biomarkers such as ALT and ALP. These biomarkers provide a tool for specific early warning sign for aquatic pollution also in fish species (Strmac and Braunbeck, 2000).

The activity of Alanine aminotransferase (ALT) levels can be used to measure finfish response to toxins, malnutrition, disease and other stressors. Some studies have shown that various forms of stress can cause an increase in plasma ALT activities in fish, therefore some believed that ALT would also be an important biochemical parameter for fish under stress (De Smet & Blust, 2001). Nevertheless, the present study indicated that plasma activity of ALT increased significantly at 3 hr, and then reached the highest level at 24 hr after exposure to acute high-temperature stress and never

returned to pre-treatment levels compared with levels before even in the control group. The results of liver histology supported this finding after exposure to acute high-temperature stress, the liver of Bighead carp showed necrosis was fully damaged (Aboka et al., 2017). Necrosis is a primary reason for the increased level of ALT enzyme in the liver, which is indicative of the stress induced by heat shock. Our results are in agreement with those reported earlier in *Clarias gariepinus* exposed to different nitrite concentrations at high-temperatures (Ajani et al., 2011), tilapia after injection of benzo[α]pyrene (BaP) (Martinez-Porchas et al., 2011), and Nile tilapia (*Oreochromis niloticus* L.) under high density stress (Hegazi et al. 2014).

Alkaline phosphatase (ALP) on the other hand, is membrane-associated glycoprotein which is produced in the liver and plays a role in transport of ions and absorption of water across cell membranes. It is used as diagnostic tool for evaluation of liver function and its activity depends on fish age, maturation stage, water pollution, food composition, temperature and peculiarities of fish biology and ecology (Mehdi et al., 2011). In this experiment, Plasma ALP activity decreased at 3hr and shows no significantly different compared to the control group. It then increased and reached a maximum value at 24 hr during acute high- temperature stress. Similar results have been observed in the *Clarias gariepinus* exposed to nitrite at different water temperature (Ajani et al., 2011), and Rosy barb (*Puntius conchonius*) exposed to mercuric chloride (Gill et al., 1990).

Glucose is continuously required as energy source by cells and tissues. The level of glucose in blood plasma is maintained through the conversion of hepatic glycogen. It depends on fish physiological status, season, maturation stage, sex and environmental factors such as food consumption, water temperature, etc. Glucose levels vary widely in various species and have shown to increase at stress conditions (Mehdi et al., 2011). The production of glucose with stress assists the organism by providing energy substrates to tissues such as brain, gills and muscle in order to cope with increased energy demand. In the current study, the level of plasma glucose content increased significantly at 3 hr then reached the highest levels at 6 hr, and thereafter tended to decrease towards to the level before challenge when fish were under acute high-temperature stress. Result of this study suggest that stress has some instantaneous effect on blood glucose and when the stress lasted for a while the blood glucose could recover back to the original level (Hsieh et al., 2003). Similar results have been observed in gilthead sea bream (*Sparus aurata*) under short term crowding stress (Ortuño et al., 2001), Atlantic cod (*Gadus morhua*) under heat stress (Pérez-Casanova et al., 2008) and Wuchang bream (*Megalobrama amblycephala*Yih) under high-temperature stress (Ming et al., 2012).

The ability of fish to adapt stressful conditions is a function of genetic factors, nutritional conditions and developmental stages. Plasma cholesterol (TC) and Triacylglycerol (TG) are chiefly derived from lipid absorption in the intestines and liver fatty acid metabolism (Di Marco et al., 2008); their levels in blood plasma have been associated with stress (Pérez-Casanova et al., 2008). In this experiment, plasma TC activity decreased during acute high-temperature stress at 3hr compared to the control group. It then increased and reached a maximum value at 6hr before decreasing again. This is supported by results reported in Wuchang bream *Megalobrama amblycephala* under high-temperature stress (Liu et al., 2012). Vijayan et al. (1990) reported a reduction in triglyceride level when brook charr (*Salvelinus fontinalis*) was exposed to a stressful situation that triggered higher energy demand. Hwang and Lin (2002) found that high dosage of vitamin C could reduce liver triglyceride contents of common carp (*Cyprinus carpio*) under normal and high temperature. Likewise, Ming et al., (2012) reported that plasma TG activity showed a decreasing trend under high-temperature stress. In the present study, the plasma TG activity decreased at 3 hr during acute-high temperature stress compared to the control group. Result of this study suggest that, changes in triglyceride and cholesterol levels could be attributed to short term exposure to acute high-temperature stress and Bighead carp could have utilized substantial amount of metabolized energy in response to stressful conditions.

V. CONCLUSION

The present results show that the effect of acute high-temperatures lead to stress the fish thereby affected the physiological and biochemical responses of Bighead carp (*A. nobilis*). Increased temperatures lead to decrease the levels of plasma cholesterol and triglyceride while alanine aminotransferase, alkalin phosphatase and glucose activities were elevated under acute high-temperature stress. The overall results of the present studies indicate that Bighead carp are highly susceptible to changes in environmental temperature and rapid intervention is required in response to temperature disruptions during aquaculture. This study expected to help aquaculturists monitor tolerable temperature and time range of

exposure to stress. Aquaculturists will have to be on guard regulating toxic elements in fish culture emanating from feed additives, pollutants and hormones.

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