Effect Of Fasting Time And Transport Temperature On The Physiological Stress Of *Megalobrama Amblycephala*

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Abstract: In order to determine optimum fasting time and transporting temperature, blood physiology and water ammonia nitrogen content of *Megalobrama amblycephala* simulation different transportation conditions were examined in summer. The results showed: temperature was dropped from 26 °C to 18 °C and 12 °C, *Megalobrama amblycephala* serum cortisol (COR), glucose (GLU), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) kept higher level, fish was appeared stress response. In the 18 °C transport compared with 12 °C and 26 °C, serum ALT activity rise of fish was delayed. Fasting influence physiologies of *Megalobrama amblycephala* were little in transport process, fasting 36h merely to mitigate deterioration of water quality. The results demonstrated that temperature on physiological effects of *Megalobrama amblycephala* was main function in transport process, better temperature and fasting time of *Megalobrama amblycephala* summer transport were 18 °C and 36 h.

1. Introduction

Aquaculture facilities aging and outdated equipment, well-bred breeding and covering degree, frequent disease prevention and cure were focused attention by more practitioners and researchers, while fish circulate from fishpond to table was inadequate attention \(^{[1]}\). Flesh of fresh fish are uncontaminated by bacteria, and can maintain nutritional value. However, according to my investigation, more than 95% of freshwater fish sold were alive in the Shanghai, while the price of dead fish reduced about 50 percent. Relevant investigation and analysis show 10% economic value of Chinese freshwater fish is lost in circulation, 20 times as many as in the developed country \(^{[2]}\). Fish will be jumping, dodging and rollover due to catch, handling, vibration and water quality change...
during the transportation. Corresponding, fish appear stress reaction such as hypoxia, injure, hormone rise, energy metabolism changes, and cause body immune ability decline, leading to disease even death [3]. Currently, main mode of transport is water transport. According to season, growth stage of fish and transport distance, improve water quality [4] and oxygen [5], decreasing water temperature [6] and changing salinity [7] used to improve the quality of transportation.

Pathogens are more likely to breed in summer, and fish easily infected by pathogens. Although hypothermia can lower fish metabolic rate and control pathogens [8], the degree of cooling that can be applied is limited [9]. Fasting fish before transportation is a common practice in aquaculture. Fasting prior to transport serves to evacuate the gut and reduce oxygen demand and waste production [10]. There have not been studies on the effect of short-term fasting before fish transport reduces the transport stress and most analyze the effect of slaughter stress on fish quality, fish metabolism expression and fish feeding. Despite in China’s code of practice for live fish transportation suggest that freshwater fish should be fasted about 1 to 2 days before transportation.

However, *Megalobrama amblycephala* (Wuchang bream) is a major species of conventional freshwater fish cultured in China. There are no specific study on the optimal temperature and fasting time of fish in summer transport. Therefore, in this study we aimed to analyze the impact of short term fasting (12 h, 24 h, 36 h) and temperature (26 °C, 18 °C and 12 °C) of transport on several hematologic stress and water quality indicators to obtain the best transport conditions of *Megalobrama amblycephala*.

2. Materials and methods

2.1 Fish material and experimental design

*Megalobrama amblycephala* were purchased from a local market, and used 400 L plastic tank (control the transportation temperature is 20 °C, density of 10 fish per 100 L, provide for adequate oxygen) to the laboratory. Fish were fed in a holding pond for about 10 days (26 °C), fed for 8 days, change the water every 2 days, received daily light to restore fish to the optimum physiological state before the study. Chose the samples in the same size as test object that average weight was 551.15 ± 76.12 g, length was 33.46 ± 5.46 cm.

After transport stress elimination, *Megalobrama amblycephala* were fasted in 12 h, 24 h and 36 h, respectively. Then cooling down to 18 °C, 12 °C (the pretest showed *Megalobrama amblycephala* appear death after 10h under 12 °C), respectively. There are 9 experimental groups, 400 L plastic tank packed 30 fishes of each group, 270 fish in total in the test. The samples were tested for simulating the vibration of transportation for 12h (90 km·h⁻¹) by LX-100VTR simulation of transport shaking tester (Shanghai lu xuan instrument equipment factory), transport density was 50g/L (1 fish·10L⁻¹). Blood samples were taken from each group 5 fish at transport 0 h, 1 h, 12 h, and recovered 12 h after transport (recovered at 26°C), respectively. Water was taken from tank at transport 0 h, 1 h and 12 h, respectively. The index measure standard is fasting 12h, transport 24 °C at 0 h (control). Survival rate was tested after transportation (remaining 10 fish in each group) at 12h, 1d, 2d, 3d, 4d, 5d after transportation. The specific test group was shown in table 1.

| Fasting time | Transport temperature |
|--------------|-----------------------|
|              | 26 °C (room) | 18 °C | 12 °C |
| 12h          | F₁T₁ (control) | F₁T₂ | F₁T₃ |
| 24h          | F₂T₁         | F₂T₂ | F₂T₃ |
| 36h          | F₃T₁         | F₃T₂ | F₃T₃ |

*Megalobrama amblycephala* was anesthesia death by 200mg/L MS-222 for 2 min, fish weight and length were been measured. Blood was collected from the caudal vasculature using a 1-mL disposable syringe and kept in 2-mL centrifugal tube for 2 h at 4 °C before centrifugation for 5 min at 10,000 rpm. The collected serum was kept at -80 °C refrigerator (Haier DW-86L288, China) for later
analysis of cortisol, glucose, and enzymatic activity. Transport water was collected from the tank using 250 ml sterile bottle and sealed at 0 °C refrigerator (Haier BCD-216WMPT, China)

2.2 Serum cortisol and glucose
Serum cortisol was determined by means of an enzyme-linked immunoassay according to the Fish Cortisol ELISA Kit manufacturer’s instructions. Serum glucose was determined by means of the glucose oxidase method according to the instructions of a commercial Glu testing kit.

2.3 Enzyme assay
Alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate transaminase (AST) were determined using commercial analysis kits (Nanjing Jiancheng Biological Engineering Institute, China) according to the manufacturer’s instructions.

2.4 The total ammonia nitrogen of water
The content of ammonia nitrogen was determined on the water flow analyzer (SKALAR San++, Netherland) after the transport water was filtered by 0.22um ultrafiltration membrane.

2.5 Statistical analysis
Analysis were replicated five times (n=5). The design was completely randomized. Results were reported as mean values of each determinations ± standard deviation (SD). Analysis of variance and significant difference tests were performed by the Duncan (D) multiple comparative method (SPSS version 19.0). The significance was defined at \( P < 0.05 \). Different uppercases are significantly different in different treatment groups of same time. Different lowercases are significantly different in different times of same treatment group.

3. Results and discussion

3.1 Changes of serum COR concentration of Megalobrama amblycephala
When fish is influenced by fishing, carrying, vibrating, changing of water quality and temperature, it leads to the change of hormone levels mediated by the neuroendocrine system firstly. COR is a kind of hormone produced by adrenal glands. The increase of blood COR concentration is considered to be a sensitive signal of animal stress [11-13]. The serum COR changes before and after the transportation under different fasting time and temperature conditions were showed in Table-2.

Transport 0h, serum COR concentration of the same temperature groups has no obvious difference (\( P > 0.05 \)), shown that there is no stress response in Megalobrama amblycephala during fasting for 12h, 24h and 36h. Lopez [14] claim that blood COR concentration of rainbow trout after short-term fasting for 3d has no change compared with the normal feeding groups. At 12°C and 18°C transport, serum COR concentration of the same fasting time groups was significantly higher than 26°C (\( P < 0.05 \)), that shown Megalobrama amblycephala had obvious stress response in order to adapting low temperature. Vanlandeghem [15] found blood COR levels of micropterus salmoides rose significantly, when micropterus salmoides transported from 20°C to 8°C and stayed 1h later. The serum COR concentration was significant increase (\( P < 0.05 \)) after transport 1h in each groups compared with transport 0h. It confirmed that the blood COR concentration increased rapidly when the fish subjected to transportation stress [16-18]. After transport 12h, Serum COR of Megalobrama amblycephala in 18°C and 12°C were lower than 26°C (\( P < 0.05 \)), and has no difference compared with transport 1h (\( P > 0.05 \)), illustrate that fish at 18°C and 12°C can be better adapted to transportation stress. Garcia [19] reported that milkfish larvae under the transportation of 20°C has significantly higher survival rate than 28°C.

Transport recovery 12h, serum COR of high temperature groups (F1T1, F2T1, F3T1) were higher than other low temperature groups (\( P < 0.05 \)), and unable to return to the pre-transport concentration.
levels ($P < 0.05$), while low temperature groups compared with transport 0h groups has no significant difference ($P > 0.05$). That showed *Megalobrama amblycephala* in 26 ℃ transportation could cause relatively strong stress reaction, while *Megalobrama amblycephala* could better adapt to transportation stress in low temperature. The same temperature and transportation time groups, COR level were no difference between different fasting time groups ($P > 0.05$), showed that short-term fasting had no influence for *Megalobrama amblycephala* to relieve stress.

Table-2 Changes of serum COR(ng·ml⁻¹) in *Megalobrama amblycephala*

| Groups     | Transport 0h     | Transport 1h     | Transport 12h    | Recovery 12h    |
|------------|------------------|------------------|------------------|-----------------|
| F₁T₁       | 32.61±2.45<sup>αa</sup> | 58.92±3.32<sup>αb</sup> | 78.43±2.39<sup>αc</sup> | 50.33±3.95<sup>βb</sup> |
| F₁T₂       | 37.78±3.12<sup>βa</sup> | 58.09±1.97<sup>αb</sup> | 59.50±2.51<sup>βb</sup> | 36.61±2.87<sup>αa</sup> |
| F₁T₃       | 47.76±2.78<sup>γa</sup> | 62.50±3.87<sup>αb</sup> | 61.83±1.79<sup>βb</sup> | 40.98±3.45<sup>αa</sup> |
| F₂T₁       | 30.18±2.05<sup>αa</sup> | 60.04±4.85<sup>αb</sup> | 76.11±3.35<sup>αc</sup> | 49.39±2.33<sup>βd</sup> |
| F₂T₂       | 38.43±2.16<sup>βb</sup> | 59.02±3.47<sup>αb</sup> | 58.63±2.68<sup>βb</sup> | 42.28±3.85<sup>αa</sup> |
| F₂T₃       | 45.96±3.45<sup>γa</sup> | 62.18±3.12<sup>αb</sup> | 61.43±3.24<sup>βb</sup> | 40.59±3.56<sup>αa</sup> |
| F₃T₁       | 31.93±3.36<sup>αa</sup> | 60.87±2.89<sup>βb</sup> | 79.39±3.49<sup>αc</sup> | 47.28±3.61<sup>βd</sup> |
| F₃T₂       | 37.63±3.01<sup>βa</sup> | 59.89±2.75<sup>αb</sup> | 58.43±4.05<sup>βb</sup> | 36.26±2.82<sup>αa</sup> |
| F₃T₃       | 49.96±3.45<sup>γa</sup> | 61.11±2.32<sup>αb</sup> | 60.31±3.25<sup>βb</sup> | 46.13±3.11<sup>βa</sup> |

3.2 Changes of serum GLU concentration of *Megalobrama amblycephala*

Blood glucose is an important energy source in animals, which can directly reflect the metabolism of fish, the changes of *Megalobrama amblycephala* blood glucose concentration in different fasting time and temperatures transportation are shown in Table-3.

Transport 0h, serum GLU concentration of *Megalobrama amblycephala* in 12 ℃ and 18 ℃ were obviously higher than 26 ℃ ($P < 0.05$) in the same fasting time and different temperature groups. It is due to the fact that physiological changes were produced by fish to resist external stimuli when the temperature was reduced, gluconeogenesis and glycogenolysis were caused due to the rise of COR to satisfy body’s demand for energy under the condition of the stress, leading to higher level of GLU [20, 21]. In the same temperature and different fasting time groups, serum GLU levels have no apparently difference with the extension of fasting time. Li Qin [22] found that blood COR and GLU concentration of *pelteobagrus vachelli* larvae has no difference compared with control group after fasting 1h. Transport 1 h, serum GLU of F₁T₁, F₂T₁ and F₃T₁ groups were higher than transport 0 h ($P < 0.05$). While GLU of low temperature treatment groups transport 1h were no difference than transport 0 h ($P > 0.05$). It that indicated the condition of low temperature transportation could lead to the rise of COR level, nevertheless reduced transportation stress, which was due to the rate of respiratory metabolism rate and the amount of exercise are decreased under low temperature condition. Transport 12 h, serum GLU of low temperature groups were no significant difference than transport 1h ($P < 0.05$), while it had remarkable increase than transport 1h in normal temperature group ($P < 0.05$). Dhanasiri [23] reported that the related genes of glycolysis and glycolysis of Zebra Fish were induced and expressed, which lead to blood GLU concentration increased significantly under the condition of normal temperature and 48h packing transport. After 12h transport recovery, the GLU level of each treatment groups have returned to the level of F₁T₁ (pre-transport), indicated that the metabolic rate was return to normal. By comparing the GLU level in same temperature and different fasting time, it was found that no obvious effect of short-term fasting on metabolic regulation in transportation process at each stage of transportation.
Table-3 Change of serum GLU (mmol•mL⁻¹) in *Megalobrama amblycephala*

| Groups | Transport 0h | Transport 1h | Transport 12h | recovery 12h |
|--------|--------------|--------------|---------------|--------------|
| F₁T₁   | 6.05±0.43    | 8.95±0.12    | 13.45±0.88    | 6.75±0.67    |
| F₁T₂   | 9.40±0.25    | 10.68±0.83   | 10.33±0.95    | 9.90±0.41    |
| F₁T₃   | 9.95±0.51    | 9.65±0.94    | 9.89±0.86     | 9.61±0.69    |
| F₂T₁   | 6.70±0.92    | 9.56±0.25    | 13.98±0.94    | 8.85±0.62    |
| F₂T₂   | 10.17±0.65   | 9.19±0.86    | 9.63±0.52     | 6.03±0.63    |
| F₂T₃   | 10.35±0.53   | 10.54±0.79   | 10.12±0.83    | 6.56±0.27    |
| F₃T₁   | 7.02±0.78    | 10.98±0.77   | 14.51±0.69    | 6.01±0.99    |
| F₃T₂   | 9.52±0.89    | 10.01±0.89   | 10.56±0.64    | 6.86±0.65    |
| F₃T₃   | 10.98±0.93   | 12.46±0.96   | 11.23±0.75    | 6.59±0.82    |

3.3 Changes of serum ALT and AST activities in *Megalobrama amblycephala*

AST and ALT are mainly existed in liver, which play a key role in amino transfer in amino acid metabolism, which is an important transaminase of fish. They are released into the bloodstream when liver cells are damaged. Therefore, the increase of serum AST and ALT activities is considered to be the most specific and widely used indicator of liver function injury [24]. The changes of serum ALT and AST activities in different treatment groups were shown in Table-4 and Table-5 respectively.

Transport 0 h, The serum ALT and AST activities of *Megalobrama amblycephala* under the 12℃ transport condition were greatly higher than 18℃ and 26℃ (P <0.05), indicated that low temperature would damage the liver of fish. Guan [25] found that blood ALT and AST activities of rainbow trout increased remarkably after 2h under low temperature (6℃) stress. After transport 1h, the ALT activity of all treatment groups had no change (P >0.05) compared with the pre-transport groups, while the AST activity of all treatment groups were obvious increased compared with F₁T₁ (P <0.05), indicated that transportation stress would still damage the liver of *Megalobrama amblycephala*. The AST activity of the samples transported at 18℃ was significantly lower than 26℃ and 12℃ (P <0.05), showed that the liver injury was minimal in short time transportation at 18℃. Transport 12 h, serum ALT and AST activities of each group were significantly higher than transport 0 h and 1 h (P <0.05), while serum ALT activity of transport at 18℃ was significantly lower than 26℃ and 12℃ (P < 0.05). It indicated that liver tissue was damage aggravated by long-term transportation stress, however, the increase of ALT would be delayed for a long time transport at 18℃. After transport 12h of normal temperature recovery, the serum AST activity of each group returned to normal level basically, but the activity of ALT was remarkable higher than normal level (F₁T₁)(P<0.05), showed that the liver of *Megalobrama amblycephala* was irreversibly damaged after the recovery of transportation. This may be one of the reasons of death in all groups after transport 12h.

Table-4 Change of serum ALT (U·L⁻¹) activity in *Megalobrama amblycephala*

| Group | Transport 0 h  | Transport 1 h  | Transport 12 h | recovery 12 h |
|-------|---------------|---------------|---------------|--------------|
| F₁T₁  | 29.56±1.36    | 30.59±1.89    | 66.89±3.85    | 40.69±3.06   |
| F₁T₂  | 29.89±1.98    | 32.86±2.11    | 57.66±4.54    | 36.76±2.53   |
| F₁T₃  | 36.63±1.21    | 37.33±2.75    | 67.75±3.28    | 40.98±1.84   |
| F₂T₁  | 30.16±1.82    | 29.78±2.66    | 65.96±2.78    | 42.75±2.89   |
| F₂T₂  | 30.69±1.66    | 30.97±1.79    | 54.74±3.65    | 40.94±1.53   |
| F₂T₃  | 37.96±1.72    | 38.88±1.22    | 68.69±4.52    | 40.69±1.65   |
| F₃T₁  | 29.69±2.31    | 30.61±2.61    | 67.72±5.20    | 39.85±2.29   |
| F₃T₂  | 30.77±1.73    | 31.23±2.97    | 58.77±4.71    | 40.26±2.79   |
| F₃T₃  | 38.63±2.06    | 37.81±1.24    | 69.26±6.01    | 42.36±3.33   |
Table-5 Change of serum AST (U·L⁻¹) activity in *Megalobrama amblycephala*

| Group | Transport 0 h | Transport 1 h | Transport 12 h | Recovery 12 h |
|-------|---------------|---------------|----------------|--------------|
| F1T1  | 46.44±2.32⁻³  | 64.34±2.78⁻³  | 92.59±3.87⁻³  | 46.69±3.17⁻³  |
| F1T2  | 47.43±2.98⁻³  | 55.66±2.54⁻³  | 93.86±3.11⁻³  | 47.86±2.93⁻³  |
| F1T3  | 59.63±3.23⁻³  | 67.38±2.28⁻³  | 94.33±3.75⁻³  | 48.98±2.94⁻³  |
| F2T1  | 45.56±2.96⁻³  | 63.80±2.38⁻³  | 90.78±2.66⁻³  | 46.75±2.09⁻³  |
| F2T2  | 43.68±2.37⁻³  | 54.74±1.62⁻³  | 89.97±2.23⁻³  | 46.94±2.87⁻³  |
| F2T3  | 56.96±2.02⁻³  | 66.82±2.02⁻³  | 91.46±3.28⁻³  | 47.69±3.25⁻³  |
| F3T1  | 47.69±2.32⁻³  | 67.72±2.20⁻³  | 92.61±2.61⁻³  | 48.85±2.09⁻³  |
| F3T2  | 46.79±1.73⁻³  | 56.71±1.87⁻³  | 88.23±3.97⁻³  | 46.26±3.74⁻³  |
| F3T3  | 62.63±2.87⁻³  | 78.26±2.71⁻³  | 91.01±3.24⁻³  | 50.36±2.01⁻³  |

3.4 Change of water total ammonia nitrogen content

Fish excreta contain a large amount of ammonia nitrogen. Free ammonia (NH₃) and ammonium ion (NH⁴⁺) were the mainly form of ammonia nitrogen in water, NH₃ is the main harmful to fish, studies have shown that NH₃ can enter fish body through lipid biofilm and hurt the gill epidermis cells of fish. Meanwhile, the antioxidant system of fish also affected, resulting in increase in lipid peroxidation products, thereby reducing the immunity of fish [26, 27]. The changes of total ammonia nitrogen content in transport-water under different fasting time and temperature shown in Table-6.

After transport 1h, the total ammonia and nitrogen content in transport-water of each group were increased greatly (P< 0.05), illustrated that transportation stress would cause fish discomfort and increase excreta. However, the total ammonia and nitrogen in water in fasted 36h groups compared with fasted 12h and 24h groups were decreased obviously (P < 0.05), the same as transport at 12°C and 18°C compared with 26°C (P < 0.05). It proved that long time fasting and low temperature transportation could reduce ammonia nitrogen content, which is because low temperature and long-time fasting could reduce the metabolic rate of fish and then delay the deterioration of transport water quality. After transport 12h, the total ammonia nitrogen content in the transport-water of each group increased continuously, indicated that *Megalobrama amblycephala* still affected by the transportation stress.

Table-6 Change of water total ammonia nitrogen content (mg/L)

| Group | Transport 0 h | Transport 1 h | Transport 12 h |
|-------|---------------|---------------|--------------|
| F1T1  | 3.17±0.10⁻³  | 4.58±0.09⁻³  | 6.67±0.12⁻³  |
| F1T2  | 2.14±0.13⁻³  | 3.26±0.12⁻³  | 4.39±0.08⁻³  |
| F1T3  | 2.08±0.06⁻³  | 3.34±0.15⁻³  | 4.39±0.08⁻³  |
| F2T1  | 3.26±0.16⁻³  | 4.39±0.08⁻³  | 6.67±0.12⁻³  |
| F2T2  | 2.05±0.11⁻³  | 3.39±0.09⁻³  | 4.39±0.08⁻³  |
| F2T3  | 2.20±0.07⁻³  | 3.26±0.14⁻³  | 4.39±0.08⁻³  |
| F3T1  | 2.36±0.10⁻³  | 3.33±0.10⁻³  | 4.39±0.08⁻³  |
| F3T2  | 1.56±0.08⁻³  | 2.26±0.13⁻³  | 4.39±0.08⁻³  |
| F3T3  | 1.66±0.09⁻³  | 2.19±0.06⁻³  | 4.39±0.08⁻³  |

4. Conclusion

After fasting 12 h, 24 h and 36 h, serum COR, GLU, AST and ALT levels of *Megalobrama amblycephala* had no obvious change, and could not play a key role in the ease transport stress, but after 36 h fast can effectively relieve the deterioration of water quality of transportation. Temperature drop to 12 °C, serum COR, GLU, AST and ALT levels of *Megalobrama amblycephala* were increased significantly and led to acute stress, while increasing of serum COR, GLU concentration and water ammonia nitrogen content were slow down at 12°C transport, however, serum ALT and AST
could not effectively slow. Temperature was reduced to 18 °C, COR and GLU of *Megalobrama amblycephala* were increased, however, liver tissue of fishes were not damaged. Fishes were transported long time at 18 °C that had a certain effect to delay serum ALT continue to rise, and also effective remitted deterioration of water quality.

In summer, suitable transport conditions of *Megalobrama amblycephala* was at 18 °C after fasting 36 hand could guarantee the transportation quality. Also according to actual needs, at lower densities could be appropriate to reduce fasting time.

**Acknowledgments**

We are grateful to the Major science and technology projects of Henan (161100110600, 161100110900), Key science and technology project of Henan (182102110331) and Doctoral Research Foundation of Zhengzhou University of Light Industry (2016BSJJ023).

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