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Acute Thermal Stress Reduces Skeletal Muscle Growth and Quality in Gibel Carp (*Carassius gibelio*)

Qixin Hu [1], Jiamin Lu [1], Yu Yang [1], Dapeng Li [1,2,3,*] and Jieya Liu [1,4,*]

[1] College of Fisheries, Hubei Hongshan Laboratory, Huazhong Agricultural University, Wuhan 430070, China; huqixin@webmail.hzau.edu.cn (Q.H.); jiaminlu0911@foxmail.com (J.L.)
[2] Engineering Research Center of Green Development for Conventional Aquatic Biological Industry in the Yangtze River Economic Belt, Ministry of Education, Wuhan 430070, China
[3] Hubei Provincial Engineering Laboratory for Pond Aquaculture, Wuhan 430070, China
[4] College of Life Sciences and Technology, Tarim University, Alar 843300, China
* Correspondence: ldp@mail.hzau.edu.cn (D.L.); dkyliujieya@163.com (J.L.)

Abstract: An acute elevation in temperature impacts fish physiology and in turn causes an alteration in growth performance. This study investigated the effect of acute thermal stress on skeletal muscle growth and quality in gibel carp (*Carassius gibelio*). The gibel carp were randomly assigned to three temperature treatments, 20 °C, 26 °C, and 32 °C, for 168 h. The muscular quality characteristics and the expressions of the genes related to muscle growth were assessed at 0 h, 1 h, 12 h, 24 h, 72 h, and 168 h. The muscle nutrient content was significantly higher in the 20 °C treatment, and the muscle was more tender and elastic. The gene expression levels of the MRFs family were significantly upregulated and then gradually decreased after 1 h. The expression level of *MSTN-2* was increased in the 32 °C treatment at 168 h, in support of the slow growth rate under acute thermal stress. It is implied that gibel carp could adapt to acute thermal stress to a certain extent. Acute thermal stress, however, eventually led to a decrease in muscle growth rate and quality.

Keywords: thermal stress; muscle quality; muscle mass; MRFs family; gibel carp

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1. Introduction

A persistent and global issue is the rise in global water temperature brought on by the greenhouse effect [1]. Fish often face the dilemma of acute thermal stress, particularly during transport and seasonal changes. This can have negative impacts on their immune system, antioxidant capacity, muscle mass, and growth performance due to the drastic changes in their external environment [2–4].

It is universally acknowledged that fish muscle quality is influenced by a variety of inherent or external factors, such as the available nutrients, enzymes, genetics, climate, predators and so on [5–9]. Among them, stressors from the exogenous environment are often difficult to avoid during a period of factory farming or an experiment. As ectotherms, most fish live in waters with seasonal variations and, when the weather in their habitat changes suddenly and the water temperature exceeds their tolerance ability, it will have adverse consequences for the development, growth, reproduction, metabolism, and behaviour of the fish, and even lead to death [10–14]. In a cold stress study of *Epinephelus coioides*, both crude protein and crude fat deposition in muscle were found to be significantly reduced [15]. Some studies in zebrafish embryos found that acute extreme temperature treatment, whether low or high temperature, resulted in reduced aerobic performance and thermal sensitivity and a switch in muscle fibre type [16,17]. In a breeding trial with a heat stressor, it was found that the growth performance of *Cyprinus carpio* was significantly reduced after 60 days [18]. *Cyprznus carpzo* L. had the smoothest heart rate and metabolic frequency at 19.0 °C and, in the rest of the temperature conditions, both warming and cooling, sharp changes in heart rate and metabolic frequency were exhibited [19]. In

addition to muscle nutrients, texture is also an important part of evaluating the quality of fillets. The texture of fish muscle is mainly related to myofiber properties. The type of myofibril, cross-sectional area, diameter, and myofibril density determine the textural properties and colour of fish muscles. Texture includes hardness, springiness, cohesiveness, gumminess, chewiness, resilience, and shear force. In fish, the hardness and elasticity of the muscle becomes greater as the fish grows, and the meat becomes tougher and tastes better [20]. The study observed the effects of incubating zebrafish embryos in three different temperature treatment groups—22 °C, 26 °C, and 31 °C. After hatching, the embryos were then reared at a constant temperature of 26 °C. The results showed a noticeable variation in the number of muscle fibres, with the trend being 26 °C > 31 °C > 22 °C [21]. During the yolk stage, the *Dicentrarchus labrax* L. was reared at two temperatures: natural temperatures of 15 °C and 17.7 °C. Subsequently, the larvae were transferred to a normal temperature environment. Muscle growth during the yolk stage was similar in the two experimental groups. However, at 25, 80, and 120 days, the 17.7 °C treatment group exhibited greater hypertrophy and hyperplasia of white muscle fibres ($p < 0.05$) [22].

From early embryonic cells through ultimate differentiation and maturity, fish muscle fibre formation and growth is a very complicated regulatory process that is influenced by a variety of signalling pathways and positive and negative transcription factors, as well as exogenous stress at various stages [23,24]. The MRFs family encodes four muscle-specific transcription factors—myogenic determining factor (*Myod*), myogenin (*Myog*), myogenic factor 5 (*Myf5*), and myogenic regulatory 4 (*MRF4*)—which control the entire process of muscle development, including stereotypy, proliferation, and myofibril formation from precursor myoblasts to postnatal muscle maturation and functional refinement [25]. This shows that the expression of MRFs is closely related to the improvement of muscle quality. The MRFs family first activates the entire regulatory network through an activation process and the MRFs family is activated in two pathways, by itself and by each other. Among myosatellite cells, *Myf5* and *Myod* are the first to be expressed, causing myosatellite cells to differentiate into myogenic cells; *Myog* and *MRF4* mainly cause myogenic cells to switch to terminal skeletal muscle fibres [26]. *Myostatins* (*MSTNs*) are genetically encoded muscle growth inhibitor proteins that maintain the resting state of myosatellite cells by negatively regulating the cell cycle circulating [27]. In experiments with zebrafish fasting for 0, 3, 7, and 14 d, zebrafish starved for 14 days showed a significant reduction in *Myod* gene expression [28]. Mouse studies show that *MRF4* directly induces terminal differentiation of myoblasts, which is associated with muscle development, and that its absence may lead to muscle hypoplasia [29,30]. *Myog* expression level was discovered to be down-regulated at lower rearing temperatures in the study on Senegalese sole (*Solea senegalensis*), with smaller muscle fibre and a negative impact on muscle growth [31]. *Piaractus mesopotamicus* was farmed for 60 days at three different temperatures, 24 °C, 28 °C, and 32 °C. It was found that the expression of *Myod* was highest in the 24 °C treatment group on day 30 and day 60, suggesting that high temperatures may inhibit *Piaractus mesopotamicus* muscle growth [32]. In sea bass larvae, the highest levels of MRFs family and *MyHC* transcripts occurred at 8 °C but not in the 20 °C treatment group [33].

Gibel carp is a new variety selected and bred by Gui, an academic at the Chinese Academy of Sciences, and his team [34]. It has a good hybrid advantage as a hybridization of *Carassius auratus gibelio* and *Cyprinus carpiouar singuonensis*, with an obvious effect of increasing yield, and flesh is tender and nutritious [35]. The scales of gibel carp are tightly packed and not easily descaled. It is more stable in terms of genetic traits and has a low incidence of Myxobolus pharynae disease [36]. There exists a general consensus that increasing fish production is no longer the primary goal of aquaculture and that obtaining higher quality fish should be the goal [37,38].

Assessing the impact of acute thermal stress on muscle mass in gibel carp is critical since muscle is the most important and edible element of the fish from an economic standpoint. Even though there have been some studies on the effect of other environmental factors on the muscle quality of gibel carp, the association between acute thermal stress and

the expression level of the MRFs family is poorly understood in aquatic organisms. Therefore, this study revealed, for the first time, the changes in muscle quality and muscle growth factors in gibel carp under acute thermal stress. The findings provide a scientific basis for changes in the economic value and welfare of fish under high temperature conditions.

2. Materials and Methods

2.1. Fish, Experimental Design and Sample Collection

All fresh fish originated from the same batch farmed by the Institute of Hydrobiology, CAS. One-hundred-and-eighty fish were stocked in separate tanks for at least 1 week for acclimatization before the experiment. During the period of acclimatization, they were fed 2% of their body weight. All fish with an initial average weight of 141.95 ± 25.32 g per tail were evenly and randomly divided into 9 tanks. The tanks used in the experiment were 0.5 m in diameter and were divided into three treatments including a room temperature treatment—20 °C (±1.9 °C), 26 °C (±0.30 °C), and 32 °C (±0.49 °C). Heating rods with a power of 500 watts were used to control the water temperature, and the heating rate was 0.5 °C per hour, equally. The water in the tank was replaced daily by a recirculating aquaculture system with circulating water preheated to the set temperature for each experiment and the water temperature was measured every 6 h. The water in the tanks was replaced daily with circulating water preheated to the set temperature for each experiment. The experiment lasted for 168 h and no food was given during that period. Finally, the back white muscle samples of gibel carp were collected at 0 h, 1 h, 12 h, 24 h, 72 h, and 168 h, respectively. Three fish were randomly sampled from each tank at each time point. Fish were anesthetized with MS222 (Yuanye Bio. Co. Ltd., Shanghai, China); once a fish had become significantly less active in the water and was salvaged, it would be deconstructed for each sample collection. Samples of dorsal muscle were collected at 0 h, 1 h, 12 h, 24 h, 72 h, and 168 h for growth relative gene expression level analysis. Texture measurements, shear force, histological observation, and chemical analysis were performed on the back muscle samples collected at 168h only (Figure 1). Three replicates were applied in each experimental treatment.

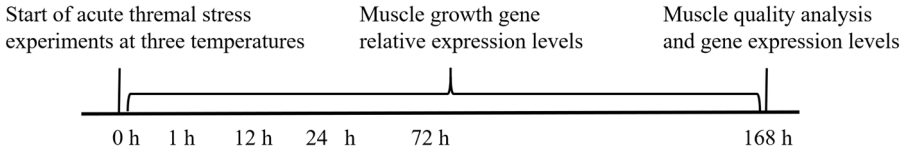

Figure 1. Sampling timeline of the acute thermal stress experiment.

2.2. Cumulative Mortality Rate and Histological Observation

The number of dead fish was recorded daily to calculate the mortality rate in the three temperature treatments. Three 1 cm × 1 cm × 0.5 cm size back muscle samples were randomly selected from the three temperature treatments and were immersed in 4% paraformaldehyde (Biosharp Bio. Co. Ltd., Anhui, China) for histological observation. Histological observation was performed based on histological paraffin section techniques and hematoxylin-eosin (H & E) staining according to the method described by Zhao et al. [39]. The diameters of back muscle fibres in three independent visual fields at different positions of each cross-section were randomly recorded, their average values were calculated for the muscle fibre diameter (μm) [40], and the distance between fibres was recorded to assess the muscle quality.

2.3. Muscle Texture Measurements

A muscle texture assay was performed as described by Lu et al. [41]. Nine fish were randomly selected from each of the three temperature treatments and two muscle samples were collected above the lateral line and below the dorsal fin, both of which were 1 cm × 1 cm × 0.8 cm in size. After collecting the back muscle samples from each culture temperature, a texture profile analysis (TPA) and a shear force test were performed

immediately at room temperature. Probe P/36R was equipped for the double compression TPA test, the post-test speed was 1 mm/s, the test speed was 1 mm/s, and the test strain was 65%. The highest peaks were manually selected after the determination of the TPA test and calculated the indicators of hardness, springiness, cohesiveness, gumminess, chewiness, and resilience. The type of loading probe was Auto-5 g for the shear force test and the shear distance was set to 3 mm. The TPA test and the shear force test were run once for each filet sample and both tests were conducted at room temperature.

2.4. Chemical Analysis

Moisture was obtained after drying samples in an oven at 105 °C for 9 h. The samples were then taken out and cooled to room temperature at three hour intervals and weighed until the difference between the two weights did not exceed 2 mg. The determination of ash content was first carbonised in an electric furnace (Hanon Analytical Co., Ltd., Jinan, China) until it was smokeless, after which it was measured with a Muffle Furnace (Hanon Analytical Co., Ltd., Jinan, China) at 550 °C for 9 h. The concentration of crude protein was measured using the Kjeldahl method, which determines nitrogen content (N × 6.25) using a Tecator Kjeltec 8400 distillation unit (FOSS Analytical Co., Ltd., Suzhou, China) after sample digestion. The content of crude lipid was determined gravimetrically using the Soxhlet method. The fishmeal was wrapped in filter paper and cotton thread before being weighed, and the dried fishmeal was extracted with anhydrous ether (Yuanye Bio. Co., Ltd., Shanghai, China) and weighed after extraction.

2.5. RNA Extraction and RT-qPCR

The total RNA of the back muscles was extracted using Trizol Reagent (Takara, Tokyo, Japan). The concentration and purity of the RNA was evaluated using a Nano Drop 8000 Spectrophotometer (Nano Drop, Boston, MA, USA) and agarose gel electrophoresis, respectively. Reverse transcription was performed when the results of the Nano Drop 8000 Spectrophotometer showed that the values of A260/A280 and A260/A230 were both greater than 2.0, and when agarose gel electrophoresis band images were clear and bright, indicating reliable RNA quality. The total RNA was used as a template for reverse transcription using the Hifair® III 1st Strand cDNA Synthesis Super Mix for qPCR (gDNA digester plus) kit (YEASEN). Primers of *Myog*, *Myod*, *MRF4*, *MSTN-1*, and *MSTN-2* genes were synthesized by Wuhan Tianyihuiyuan Biotechnology Co. (Wuhan, China) and are presented in Table 1. All genes for which relative quantification was performed were normalized with *β-actin*. All experiments were performed in triplicates.

Table 1. The primer sequences for genes cloning and expression in the study of gibel carp.

Primer	Forward Sequence (5′ to 3′)	Reverse Sequence (5′ to 3′)
Myog	GGACGCACTGCTCCACTCTG	AGGAACATCAGCAGGGAAACC
Myod	GAGAGCATCCAGAGGGCATC	AGTTCTACCTGGCCTCCAGT
MRF4	CTGCGACGGTCAGTGTCTAATGT	CAGCCTCTGGTTCGGATTGG
MSTN-1	TCAGTCCGAAGATCCAAGCG	TCCTGCGTTCACGTCGATTT
MSTN-2	TGCATGCCATCAAGTCCCAA	TCATCCCCCAGAACGTCGTA
β-actin	CATTGACTCAGGATGCGGAAACT	CTGTGAGGGCAGAGTGGTAGACG

The expression results were calculated by using the $2^{-\Delta\Delta CT}$ method and normalization after verification that the primers amplified with an efficiency of approximately 100%.

2.6. Statistical Analysis

The experimental data were expressed as mean ± SE, using a one-way variance analysis (ANOVA) and a *t*-test in SPSS 22.0 (IBM, Armonk, NY, USA); the results of the statistical analysis were deemed significant at an alpha level of 0.05. For the quantitative statistics of the histological observation, the mapping software Image J 1.53k was employed (NIH, Bethesda, MD, USA). For each gene (*Myog*, *Myod*, *MRF4*, *MSTN-1*, *MSTN-2*), data

from qRT-PCR were optimized by comparative Ct ($2^{-\Delta\Delta CT}$) value to compute the relative gene expression level in the three temperature treatments of gibel carp [42]. For histological observation and gene expression level tests, asterisks (*) indicate statistical significance compared to the control group, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. As for muscle textural analysis, different superscripts were used to show the significance ($p < 0.05$).

3. Results

3.1. Cumulative Mortality Rate and Structure of Muscle Fiber of C. gibelio

During the acute thermal stress trial, six fish perished in the 32 °C treatment, representing a 10% mortality rate, while no fish perished in the other temperature treatment groups.

Figure 2A exhibited the H & E staining sections of the white dorsal muscle of gibel carp under different water temperature treatments. Figure 2B,C showed the quantitative analysis of the images in Figure 2A. The analyses indicated significantly higher muscle fibre diameter for the 20 °C and 26 °C treatments compared to the 32 °C treatment at 168 h ($p < 0.01$). Furthermore, the average fibre interstitium in the 32 °C treatment was significantly higher than those in 20 °C treatment ($p < 0.05$).

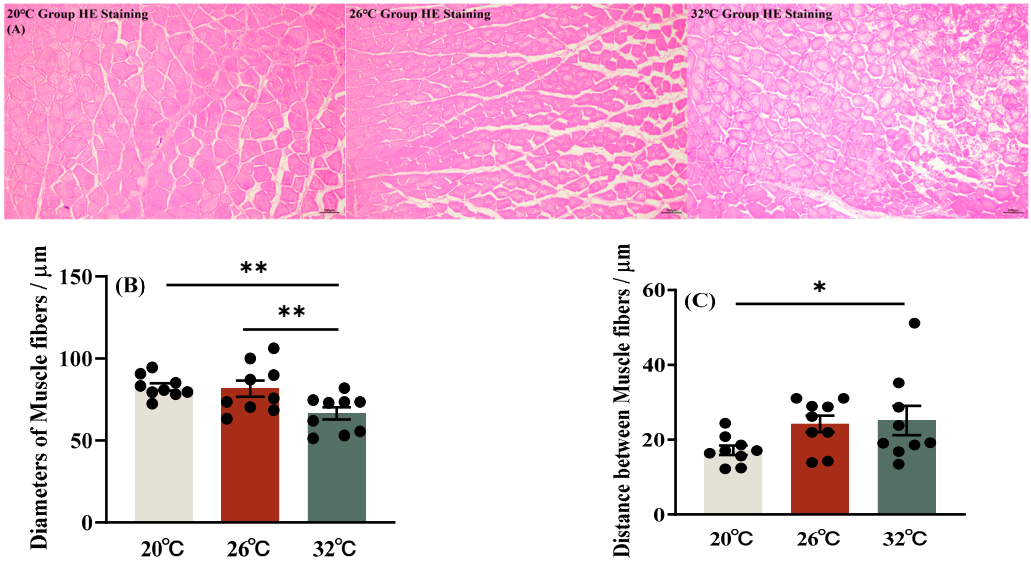

Figure 2. H & E stained sections (original magnification ×100, transverse section) of muscle tissue from gibel carp under three temperature treatments are shown in (**A**). The muscle fibre diameter and muscle fibre gap statistics under the three temperature treatments are shown in (**B**,**C**). An asterisk (*) indicates statistical significance compared to the 20 °C treatment, * $p < 0.05$, ** $p < 0.01$.

3.2. Effect of Acute Thermal Stress on Muscle Texture of C. gibelio

Measure indexes related to muscle texture are presented in Table 2. The indicators that exhibited significant differences were muscle hardness, gumminess, and chewiness, which exhibited the same trend, with the largest values occurring in the 20 °C treatment group. The 20 °C group was significantly higher than the 26 °C and 32 °C groups in the three indicators ($p < 0.05$) and also performed better at springiness, adhesiveness, cohesiveness, and resilience. The shear force was inversely proportional to the tenderness of the fillets, showing that the tenderest fillets were those treated at 20 °C. The difference between all indicators was not statistically significant in the 26 °C and 32 °C groups except for shear force.

For the eight muscle texture markers used to assess muscle composition, PCA models were created, and a degree of clustering between samples in the three temperature treatments was observed (Figure 3). PC1 and PC2 contributed 39.2% and 18.1% to the variation sources, respectively. Indicators gumminess and cohesiveness have the greatest impact on PC1 and indicators hardness and adhesiveness have the greatest impact on PC2.

The value points for the 32 °C treatment group appeared more to the left of the 0 point of PC1, whereas the value points for the 20 °C treatment appeared more to the right of the 0 point, indicating that the 32 °C group had a lower than average level of muscle texture. Consequently, the 20 °C and 32 °C treatments showed greater separation between groups due to temperature differences. Muscle texture levels were more similar in the 26 °C and 32 °C treatments.

Table 2. Comparison of eight texture indicators at three different temperature treatments of the back muscle of gibel carp.

Parameters	20 °C	26 °C	32 °C
Hardness (g)	5049.92 ± 113.16 ^{a}	4427.19 ± 72.00 ^{b}	3953.51 ± 337.20 ^{b}
Adhesiveness	−21.16 ± 1.18	−18.52 ± 1.45	−17.00 ± 4.32
Springiness	0.57 ± 0.02	0.57 ± 0.02	0.55 ± 0.04
Cohesiveness (g)	0.31 ± 0.01	0.28 ± 0.01	0.30 ± 0.01
Gumminess (g)	1565.84 ± 37.16 ^{a}	1205.91 ± 68.04 ^{b}	1207.96 ± 40.45 ^{b}
Chewiness	952.66 ± 65.41 ^{a}	722.06 ± 14.17 ^{b}	729.58 ± 47.04 ^{b}
Resilience	0.15 ± 0.01	0.14 ± 0.01	0.15 ± 0.01
Shear force	558.96 ± 4.61 ^{a}	626.76 ± 2.95 ^{b}	704.63 ± 17.14 ^{c}

Note: Different superscripts show statistical significance between groups ($p < 0.05$).

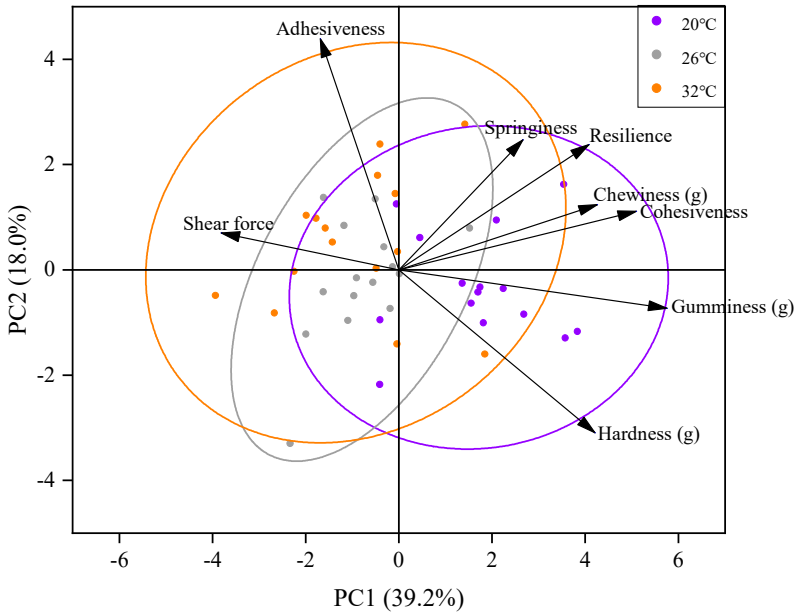

Figure 3. PCA score plots for the muscle texture indicators of gibel carp from three temperature treatments.

3.3. Muscle Nutrient Contents of C. gibelio

The fresh weight values of moisture, crude protein, crude fat, and crude ash contents are presented in order in Figure 4. Compared to groups 26 °C and 32 °C, water content was significantly lower in the 20 °C treatment group, indicating that 168 h of thermal stress reduced the content of other nutrients in gibel carp muscle. Acute thermal stress also significantly reduced protein deposition in the 32 °C treatment with a further decrease in crude fat content. A comparison of nutrient content values revealed that water concentration in the dorsal muscle increased and the protein content decreased with the increase of stocking temperature, with crude ash content exhibiting the highest value in the 26 °C treatment group.

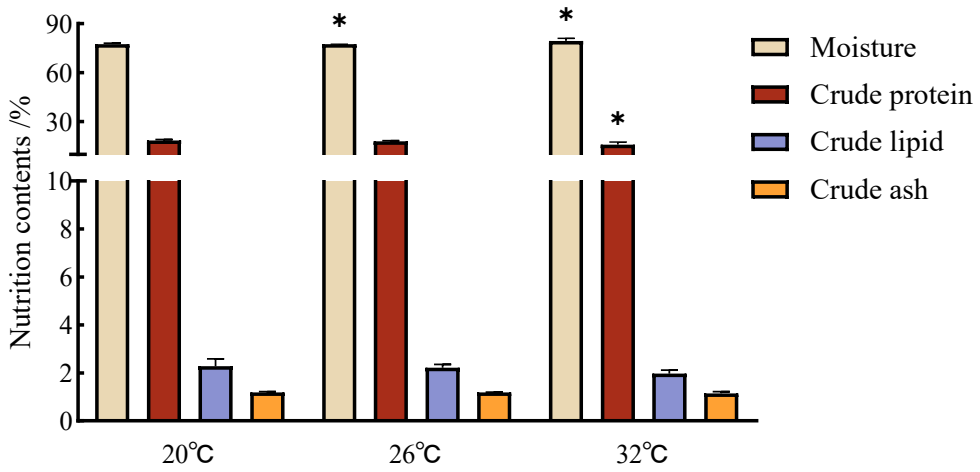

Figure 4. The nutrition contents of moisture, crude protein, crude lipid, and crude ash in white muscle of gibel carp in three temperature treatments. An asterisk (*) indicates statistical significance compared to the 20 °C treatment, * $p < 0.05$.

3.4. Effect of Acute Thermal Stress on MRFs Family Related Genes Expression Level of C. gibelio

Compared with the 20 °C treatment group, the 26 °C and 32 °C treatment groups exhibited significantly higher mRNA levels of *Myog*, *Myod*, *MRF4*, and *MSTN-1* at 1 h, whereas the expression of *MSTN-2* showed a significant increase only in the 32 °C treatment group. The peak expression of all genes appeared at 1 h, except for *Myod* and *MSTN-2*, whose peak expression appeared at 12 h after the acute thermal stress. Although the peaks appeared at different times, the results showed that all genes had a similar expression pattern in gibel carp, with an upsurge followed by a gradual decline (Figure 5). The minimum gene expression values of the positively regulatory genes associated with muscle growth all occurred at 168 h, which may indicate that the MRFs family expression and the growth of gibel carp were inhibited after acute heat stress.

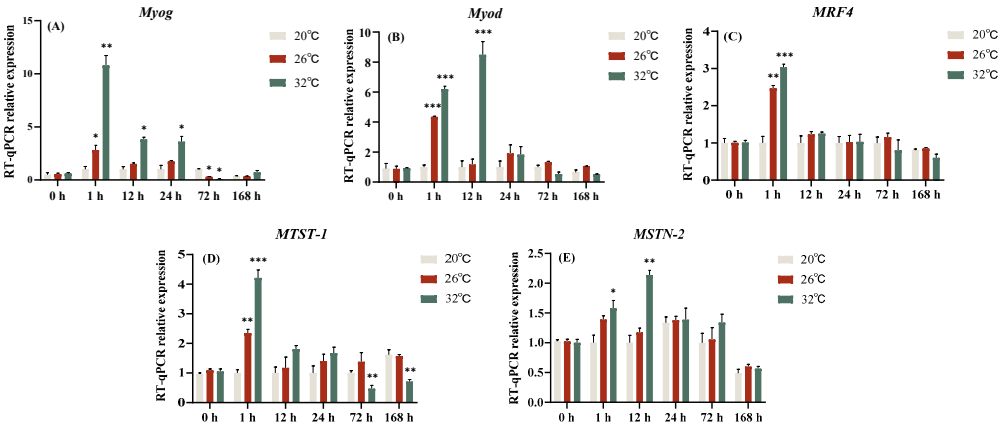

Figure 5. RT-qPCR analysis of mRNA levels of *Myog* (**A**), *Myod* (**B**), *MRF4* (**C**), *MSTN-1* (**D**), and *MSTN-2* (**E**) in white dorsal muscle of gibel carp from various temperature treatments. Gene expression levels represented the relative mRNA expression compared to the 20 °C treatment group. An asterisk (*) indicates statistical significance compared to the 20 °C treatment, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.01$.

4. Discussion

Teleost fish experience increased morbidity and mortality when water temperatures exceed their optimal range for survival due to fluctuating heating [43–45]. In our experiments, six of the sixty fish in the 32 °C treatment group died, with a mortality rate of 10%, while no mortality occurred in the other two temperature treatments, consistent with

previous studies. Thus, we speculate that the occurrence of these deaths was attributed to the difference in water temperature, because gibel carp is typically a cold-water fish and 32 °C might exceed its maximum temperature tolerance.

There is no standardized criterion for the evaluation of fish muscle quality, and people have different preferences for the texture of fish fillets [46]. Indicators such as hardness, springiness, cohesiveness, and shear force directly relate to muscle texture [47], as the results showed that the back muscle in the 20 °C treatment was softer and more tender. Changes in fillet texture are related to several factors, which include the changes in muscle fibre density, diameter, and the melting of myostromin, a major protein in connective tissue [48]. Hardness, springiness, and shear force are the main indicators of the texture of freshwater fish muscles of all the texture indicators [49,50]. It has been shown that the finer and denser the muscle, the more tender the muscle, and the better the quality of the muscle, the better the taste, and the easier it is to digest and absorb [51–53]. The shear force of the 20 °C treatment group was lower in the current study, and the hardness, gumminess, and cohesiveness of the 32 °C treatment group were significantly lower than those of the 20 °C treatment group, indicating that the muscle treated at 20 °C was more tender, had a better taste, and had a superior muscle quality. The PCA analyses of all muscle texture indexes also demonstrates that the 20 °C treatment group had the best muscle quality levels of all the temperature groups.

Muscle growth is largely dependent on the stability of protein turnover in teleost, in terms of both synthesis and degradation [54]. High temperatures above the tolerance range of the fish reduce protein deposition in skeletal muscle, which in turn slows down fish growth [55]. Researchers have different opinions about the effects of thermal stress on the protein and lipid content of livestock muscle. In studies on broilers, it has been found that thermal stress leads to a decrease in the content of some crude components in the breast muscle, which reduces the nutritional value of the broiler [56,57]. However, the opposite result was found in studies concerning pigs, where sustained thermal stress increased the content of nutrients in the muscle [58,59]. In summary, our results are similar to those of the broiler study; we speculated that acute thermal stress induced the reduction of gibel carp and might have a negative effect on protein deposition and a positive effect on moisture content.

Muscle growth in vertebrates is classified as restricted or unrestricted. In mammals, muscle growth is an increase in muscle fibre volume with no change in number, which is typical of restrictive growth [60]. Fish have both myofiber proliferation growth and hypertrophic growth. The contribution of myofibrillar growth and hypertrophic growth to fish growth depends on the species and the size of the fish [61]. Slow-growing fish, such as zebrafish, rely mostly on expanding existing myofibers rather than on producing new ones, whereas fast-growing species, such as toothfish, rely on both myofiber proliferation and hypertrophic growth [62]. The MRFs family plays a crucial role in both of these muscle growth processes. During the first process of muscle growth, the positive regulator factor, *Myod*, influences muscle fibre thickening, and *Myog* and *MRF4* affect the muscle mass and the number of muscle fibres during the second process. The negative regulators *MSTN-1* and *MSTN-2* keep skeletal muscle satellite cells in their resting state by preventing myogenic cells from cycling from G1 to S phase [63]. The expression levels of all genes in the 32 °C treatment showed a significant increase within 1 h after the start of the experiment, indicating that the gibel carp were affected by thermal stress and changed body homeostasis in 32 °C treatment, which then gradually decreased to below the initial state after 168 h. Similar results have been reported in many other animals under short-term stress, including *Siniperca chuatsi* [64], *Gadus morhua* [65], and *Oreochromis niloticus* [66]. *MSTN-1* and *MSTN-2* have the ability to inhibit the proliferation of myogenic cells, and deletion of these genes leads to an increase in skeletal muscle mass [67]. The elevated expressions of *MSTN-1* in back muscle indicated that thermal stress may contribute to the decreased muscle growth of fish. Moreover, the low mRNA expression levels of *Myod* and *MRF4* at 168 h may be due to the acute thermal stress and may lead to the subsequent slow growth of gibel carp.

5. Conclusions

Acute thermal stress causes increased mortality and decreased muscle quality and mass in gibel carp (*Carassius gibelio*). The 32 °C and 26 °C treatment groups first induced the gene expression of MRFs family and later repressed the expression, thus leading to a decrease in muscle fibre diameter and an increase in gap, ultimately causing a deterioration in taste. Compared to the stimulatory effect of the 32 °C treatment, 20 °C was optimal for muscle growth. These results provide a reference for the adverse effects of rapid climate change on muscle quality in aquaculture.

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