

Original Research Articles

Effect of chronic heat stress in roughskin sculpin (*Trachidermus fasciatus*)

Zitong Liu^{1,2,3}, Lize San^{2,3}, Zhongwei He^{2,3}, Yufeng Liu^{2,3}, Tian Han^{2,3,4}, Chunguang Gong⁴, Jiangong Ren^{2,3}, Yuqin Ren^{2,3}, Jilun Hou^{2,3a}

¹ College of Fisheries and Life Science, Shanghai Ocean University, China, ² Hebei Key Laboratory of the Bohai Sea Fish Germplasm Resources Conservation and Utilization, Beidaihe Central Experiment Station, Chinese Academy of Fishery Sciences, China, ³ Bohai Sea Fishery Research Center, Chinese Academy of Fishery Sciences, China, ⁴ Ocean College, Hebei Agricultural University, China

Keywords: Roughskin sculpin, Heat stress, Gill tissue structure, Heat stress protein

<https://doi.org/10.46989/001c.127273>

Israeli Journal of Aquaculture - Bamidgah

Vol. 77, Issue 1, 2025

The roughskin sculpin (*Trachidermus fasciatus*) belongs to the family Cottidae and is a small carnivorous catadromous fish distributed along the coasts of China, Korea, and Japan. The effects of heat stress on roughskin sculpin have yet to be systematically performed. This study aimed to explore the effects of chronic heat stress on the roughskin sculpin. The survival rate, gill histopathological changes, and heat stress protein gene expression patterns of roughskin sculpin were analyzed after different temperature stresses of 19 °C, 22 °C, 25 °C, 28 °C, and 31 °C for 0, 3, 6, 9, and 12 days. The results revealed that roughskin sculpin could not survive at 33 °C. Chronic heat stress can cause severe damage to gill tissue, reducing the gills' respiratory efficiency. Moreover, the expressions of *hspb1*, *hspb7*, and *hspb11* changed significantly after heat stress. In the 31 °C treatment group, as stress time increased, *hspb1*, *hspb7*, and *hspb11* expression initially increased and then decreased. This study's results provide a reference for the study of heat stress physiology of roughskin sculpin as well as a new idea and method for evaluating and breeding heat stress traits of roughskin sculpin.

INTRODUCTION

Temperature is an important environmental factor in aquaculture, and daily or seasonal thermal fluctuations and changes in culture water temperature may significantly affect normal physiological processes, survival, and growth of fish.¹ Global warming has also caused an increase in the water temperature in rivers and oceans. Over 90% of the excess heat accumulated in the climate system is deposited in the world's oceans, and there has been an unequivocal ocean warming trend in recent decades.² Over the past 30 years, the water temperature of rivers between 30° S and 30° N has increased the most at c. 0.5 °C per decade.³

The continuously rising temperature has caused heat stress in fish, resulting in multilevel effects, such as metabolic impairment,^{4,5} drastic decrease in disease resistance,^{6,7} alteration of the levels of various substances such as total protein and glucose, and changes in gill tissue structure.⁸ Heat stress can also change the heat shock protein and immune-related gene expression levels. The chronic heat stress up-regulated the heat shock proteins (HSP70, HSP90, GRP75, and HSC70) and inflammation factors (*IL-1 β* and *TGF- β*) mRNA expression levels in Siberian

sturgeon *Acipenser baerii* and its hybrids (*A. baerii* ♀ × *A. schrenckii* ♂).⁹ Short-term heat stress has significantly up-regulated 21 genes belonging to the HSP30, HSP40, HSP60, HSP70 and HSP90 families in Yangtze sturgeon (*Acipenser dabryanus*).¹⁰

The roughskin sculpin (*Trachidermus fasciatus*) belongs to the family Cottidae and is a small carnivorous catadromous fish that is distributed along the coastal areas of China, Korea, and Japan.^{11,12} However, the population size of roughskin sculpin has significantly declined, resulting in habitat fragmentation and local extinction due to human activities and global warming.¹¹ Since 1988, the Chinese government has listed roughskin sculpin as a Class II protected animal.¹²

Thus, the effects of heat stress on roughskin sculpin have not been systematically investigated. For the first time, we investigated the cumulative survival rate, gill histopathology, and heat shock protein gene expression in roughskin sculpin under different thermal conditions. This study's results identified the upper limit of survival temperature of roughskin sculpin and provided better strategies for protecting and culturing roughskin sculpin in the context of global warming.

a Corresponding author. Jilun Hou, e-mail: houjl@bces.ac.cn

MATERIALS AND METHODS

FISH BREEDING

The experiment was performed at Beidaihe Central Experimental Station, Chinese Academy of Fishery Sciences. The F2 generation of roughskin sculpin (1.56 ± 0.81 g in body weight and 5.53 ± 0.81 cm in body length) artificial breed at Beidaihe Central Experimental Station was used.

TEMPERATURE TREATMENT AND SURVIVAL RATE STATISTICS

A total of 150 roughskin sculpins were randomly selected and temporarily cultured for 14 days (salinity: 32, temperature: 19 °C, and DO: 7 ± 0.5 mg/L). The fish were fed normally during the temporary culture period, and the formal experiment was conducted after the stress response of the fish disappeared. Three parallel experimental groups (A1, A2, and A3) were used for the temperature experiment. The initial water temperature was 19 °C and was controlled using a constant-temperature heating rod. There were 35 roughskin sculpins in each treatment group and control group. The water temperature increased by 1.5 ± 0.2 °C each time, maintained for five days, and then continued to heat up. Isothermal seawater was used for water every 24 h, with an 80% water exchange rate. The mortality rate in each group was recorded daily. The lethal water temperature and cumulative survival rate were counted under different water temperature gradients.

STRESS AND SAMPLING

The experimental temperature gradients were set to 19 °C, 22 °C, 25 °C, 28 °C, and 31 °C, with the 19 °C treatment group serving as experimental control. Three roughskin sculpins were randomly selected from each experimental group six days after the experiment started. After anesthesia (MS-222, 70 mg·L⁻¹), the gill tissue was sampled and stored in 4% paraformaldehyde. The whole fish tissues were collected and frozen in liquid nitrogen and stored at -80 °C for 0, 3, 6, 9, and 12 days after the experiment started.

HISTOLOGICAL EXAMINATION

The fixed gill tissue was dehydrated with 70% ethanol for 45 min, followed by tissue dehydration with 80%, 90%, and 100% ethanol for 40 min after the tissue turned yellow to light. After dehydration, the samples were immersed in a mixture of xylene and ethanol at a volume ratio of 1:1 for 15 min and a pure xylene solution for 20 min until they became transparent. The tissue was immersed in a paraffin solution and embedded. The rotary slicer (Leica, Hesse, Germany) was used to slice continuously with a thickness of 5 µm. Tissue sections were dried and stained with hematoxylin and eosin. The sections were sealed with neutral gum and observed under a microscope (Olympus, Tokyo, Japan).

TOTAL RNA EXTRACTION AND CDNA SYNTHESIS

TRIzol reagent (Invitrogen, California, USA) was used to extract total RNA from whole fish tissues. The extracted RNA concentration and OD260/OD280 nm value were determined using a nucleic acid quantitative instrument (NanoDrop 2000, USA). A 1% agarose gel was prepared for gel electrophoresis to verify the RNA quality. RNA of qualified quality and concentration was selected and reverse transcribed using the gDNA Eraser primer script RT reagent kit (TaKaRa, Tokyo, Japan) to obtain cDNA.

QUANTITATIVE REAL-TIME REVERSE TRANSCRIPTION PCR (RT-QPCR)

hspb1, *hspb7*, and *hspb11* gene expression levels in whole fish tissues at different time points of high-temperature stress were detected using RT-qPCR. The 18s RNA was used as the internal reference gene. Primer Premier 5.0 was used to design primers based on the core fragment sequences of *hspb1*, *hspb7*, and *hspb11* genes (Table 1). Each assay was performed in triplicate, with each reaction mixture containing 5 µL of TB Green Premix Ex Taq II (TaKaRa, Tokyo, Japan), 0.5 µL of forward primer, 0.5 µL of reverse primer, 0.5 µL of RT PCRready cDNA, and 3.5 µL of RNase-free ddH₂O. PCR cycling conditions were 94 °C for 2 min, denaturation at 94 °C for 30 s, and extension at 60 °C for 20 s for 40 cycles. The accuracy and specificity of the PCR products were determined using a dissolution curve, and relative mRNA expression levels were calculated using the 2^{-ΔΔCt} method.¹³

STATISTICAL ANALYSIS

The experimental data were expressed as mean ± standard deviation (SD). PCR results were analyzed using fluorescence quantitative PCR instrument system software, and the relative gene expression (2^{-ΔΔCt}) was calculated. One-way ANOVA was performed using SPSS 20.0 software.

RESULTS

THE CUMULATIVE SURVIVAL RATE OF ROUGHSKIN SCULPIN UNDER HEAT STRESS

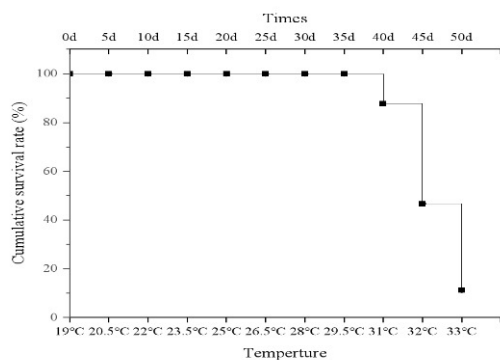
Table 2 shows that when the experimental water temperature was between 19 °C and 29.5 °C, there was no death in the experimental group, as well as no significant change in feeding, body color, or vitality. When the temperature of the aquaculture water reached 28 °C, the feeding of sculpin gradually decreased with the extension of heat stress time. At the fifth day after 28 °C treatment, $10.7 \pm 2.5\%$ of the sculpins stopped feeding, and $13.3 \pm 1.9\%$ of the sculpins had darker skin color, decreased vitality, and swimming ability. When the heat stress temperature reached 31 °C, the proportion of roughskin sculpin that stopped feeding and had a darker body color increased significantly. The number of deaths within five days was 3.67 ± 0.47 , and the mortality rate was $7.3 \pm 0.9\%$. When the heat stress temperature reached 32 °C, deaths within five days were $16 \pm$

Table 1. Gene primer synthesis sequence

Name	Sequence
ST-18S-F	TTTCGAGGCCCTGTAATTGGAA
ST-18S-R	CCGAGATCCAACCTACGAGCTTT
ST-HSPB1-F	TCCTCTGTGGAAACGACAGC
ST-HSPB1-R	GGAATGTGGCGAAGTCCTCA
ST-HSPB7-F	AACAGGTAGTCCCACTGCG
ST-HSPB7-R	GCTGTGTGTGTCAGTTGCTG
ST-HSPB11-F	GGTTGGCAGGAGCAGTAACT
ST-HSPB11-R	TGTGGTTGTGATCTCCGGTG

Table 2. Death of roughskin sculpin under heat stress

Group	19°C	20.5°C	22°C	23.5°C	25°C	26.5°C	28°C	29.5°C	31°C	32°C	33°C
A1	0	0	0	0	0	0	0	0	4	15	28
A2	0	0	0	0	0	0	0	0	3	17	26
A3	0	0	0	0	0	0	0	0	4	16	26

**Figure 1. Cumulative survival rate of roughskin sculpin under chronic heat stress**

0.82, and the mortality rate was $32 \pm 1.6\%$. When the heat stress temperature reached 33°C , deaths within five days were 26.33 ± 1.25 , and the mortality rate was $52.7 \pm 2.5\%$. Anatomical observation of the dead roughskin sculpin revealed that the skin color of the body surface was deeper than that of the normal skin, the gill cover was open, and the gill part showed a yellow-brown color with different degrees of ulceration.

When the heat stress temperature was $19^\circ\text{C} - 29.5^\circ\text{C}$, the cumulative survival rate of the sculpin was 100% (Figure 1). The cumulative survival rate of roughskin sculpin was $92.7 \pm 0.9\%$ when chronic heat stress reached 31°C . When the heat stress reached 32°C , the cumulative survival rate of the roughskin sculpin decreased to $60.7 \pm 0.9\%$. When the heat stress reached 33°C , the cumulative survival rate of roughskin sculpin decreased rapidly to $8 \pm 1.6\%$.

EFFECTS OF HEAT STRESS ON THE GILLS

Fish gills consist primarily of gill arches, rakers, and filaments. Healthy roughskin sculpin has many complete gill lamellae on both sides of each gill filament. The gill supports cartilage intact (Figure 2A). The 19°C , 22°C , and 25°C treatment groups had structurally intact gill filaments (Figure 2Aa), and intact gill support cartilage (Figure 2Ab), structurally intact gill lamellae and neatly arranged gill filaments. In the 28°C treatment group, some gill filaments were ulcerated (Figure 2Dd), and some gill lamellae were broken (Figure 2Dc). In the 31°C treatment group, the epithelial cells of the gill lamellae appeared to proliferate, and the whole gill lamellae became thicker and “rod-shaped” after proliferation (Figure 2Ee). In the 32°C treatment group, the gill filaments of roughskin sculpin were severely damaged, with some ulcerated and detached gill filaments at the ends (Figure 2Ff). In the 33°C treatment group, some gill filaments of the roughskin sculpin were completely ulcerated and detached (Figure 2Gg).

Healthy gills of roughskin sculpin (Figure 2Hh) were bright red, and the overall gill structure was intact, with clear gill filaments and lamellae arranged neatly. Under heat stress, the gill tissue of sculpin was damaged (Figure 2Hi), the color was dark red, the gill filaments and lamellae were not arranged regularly, and there was more tissue fluid around the gill filaments.

HSPB1 GENE EXPRESSION IN THE WHOLE FISH TISSUE

From 0 to 12 days of heat stress, *hspb1* gene expression in whole fish tissues was significantly different in the 31°C treatment group compared to the 19°C , 22°C , 25°C , and 28°C treatment groups ($P < 0.05$; Figure 3). The *hspb1* gene expression in the 31°C treatment group increased more than 10-fold compared to the 19°C treatment group, which increased by 14.5-fold and 15.1-fold on the 9 and 12

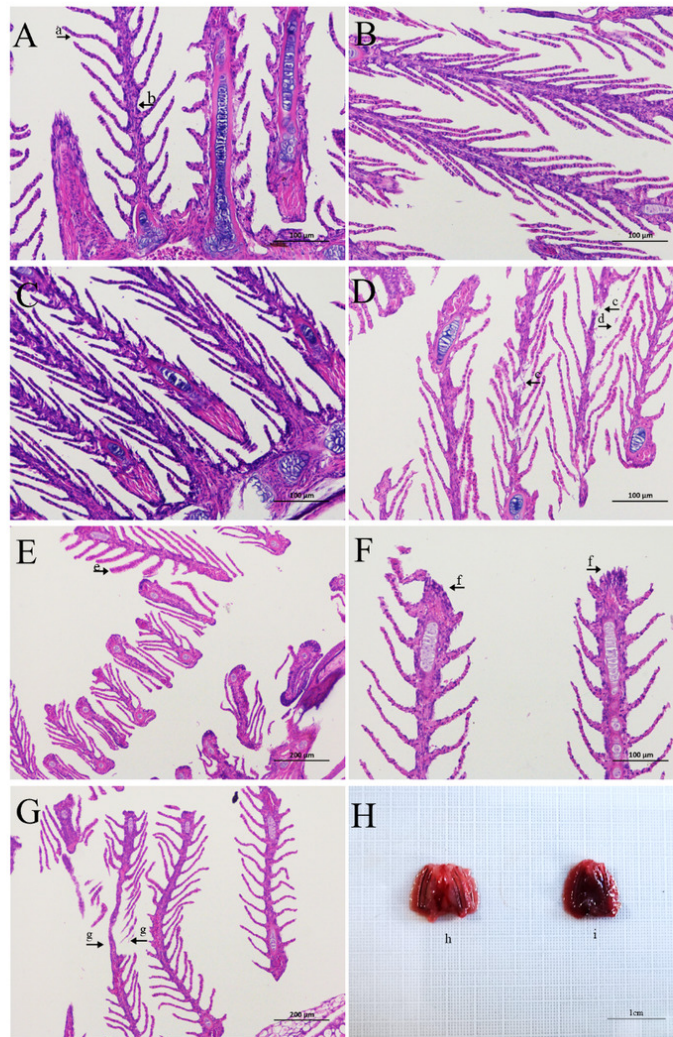


Figure 2. Microstructure of roughskin sculpin gill tissues under heat stress

A-G: Gill tissue under 19 °C, 22 °C, 25 °C, 28 °C, 31 °C, 32 °C and 33 °C stress. H: healthy and injured gill. a: Gill piece; b: Supporting cartilage; c: Gill lamellae fester; d: Gill filament fester; e: Epithelial cell proliferation of gill lamellae; f: Gill end ulceration and exfoliation; g: Gill complete fester and shedding; h: Healthy gill tissues; i: Injured gill tissue

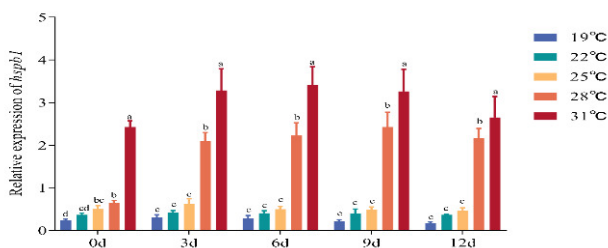


Figure 3. The relative expression level of *hspb1* in whole fish tissue of roughskin sculpin under different heat stress

days after heat stress, respectively, and the multiplicity increased with time.

hspb1 gene expression did not differ significantly between the 28 °C and 25 °C groups at 0 days ($P > 0.05$). However, as the treatment time increased, *hspb1* gene expression at 3, 6, 9, and 12 days was significantly higher in the 28 °C group than in the 25 °C group ($P < 0.05$). More-

over, the *hspb1* gene expression was significantly higher in the 28 °C treatment group than in the 19 °C and 22 °C groups from 0 to 12 days ($P < 0.05$). After 0, 3, 6, 9, and 12 days of heat stress, the ratios of *hspb1* gene expression in the 28 °C group to the 19 °C group were 2.8-fold, 6.8-fold, 7.7-fold, 10.8-fold, and 12.4-fold, respectively. The growth folds increased with time, and the difference between the two groups was the largest on the 12 days after heat stress.

The changes in *hspb1* gene expression at 0, 3, 6, 9, and 12 days of heat stress did not differ significantly between the 22 °C and 25 °C groups ($P > 0.05$; Figure 4). There was a significant difference in *hspb1* gene expression at 0 days compared to 3, 6, 9, and 12 days of heat stress in the 28 °C group ($P < 0.05$). The *hspb1* gene expression significantly increased after three days ($P < 0.05$). There was no significant difference in *hspb1* gene expression at 3, 6, 9, and 12 days ($P > 0.05$). In the 31 °C group, *hspb1* gene expression did not differ significantly between 0 days and 3, 6, 9, and 12 days after heat stress ($P > 0.05$) but differed significantly between 0 days and other time points ($P < 0.05$). *hspb1* gene expression increased significantly at three days ($P < 0.05$)

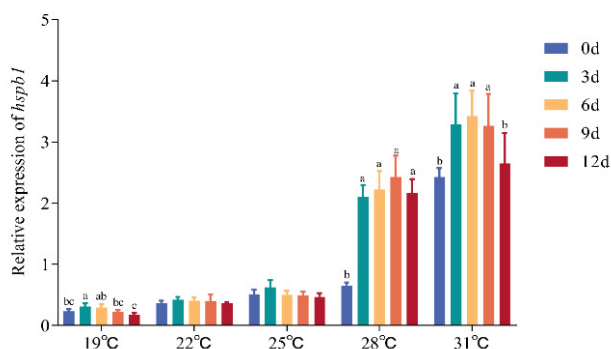


Figure 4. Changes of *hspb1* gene expression in abdominal tissue of roughskin sculpin under different temperature stress

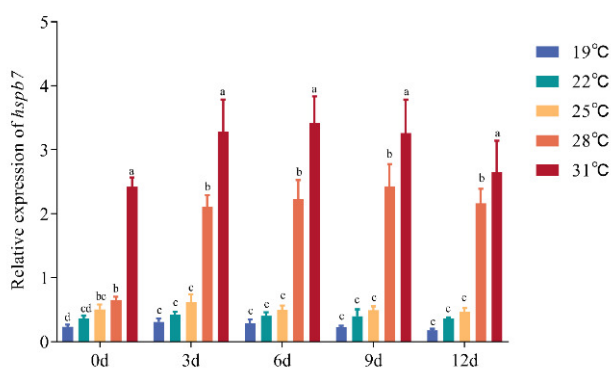


Figure 5. The relative expression level of *hspb7* in whole fish tissue of roughskin sculpin under different heat stress

and did not differ significantly between days 3, 6, 9, and 12 ($P > 0.05$).

HSPB7 GENE EXPRESSION IN THE WHOLE FISH TISSUE

From 0 to 12 days of heat stress, *hspb7* gene expression in whole fish tissue was significantly different in the 31 °C group than in the 19 °C, 22 °C, 25 °C, and 28 °C groups ($P < 0.05$; [Figure 5](#)). *hspb7* gene expression was more than five-fold lower in the 31 °C group than in the 19 °C group, and the difference was 7.8-fold after three days of heat stress, showing a trend of increasing and then decreasing.

There was no significant difference in *hspb7* gene expression after heat stress at 28 °C and 25 °C for 3 and 12 days ($P > 0.05$). However, *hspb7* gene expression in the 28 °C treatment group at 0, 6, and 9 days was significantly higher than in the 25 °C group ($P < 0.05$). Moreover, *hspb7* gene expression was significantly higher in the 28 °C group than in the 19 °C and 22 °C groups after 0 to 12 days of heat stress ($P < 0.05$). After 0, 3, 6, 9, and 12 days of heat stress, the differences in *hspb7* gene expression between the 28 °C and 19 °C groups were 3.3-fold, 3.4-fold, 3.6-fold, 4.8-fold, and 3.4-fold, respectively. The overall growth multiple initially increased and then decreased over time, with

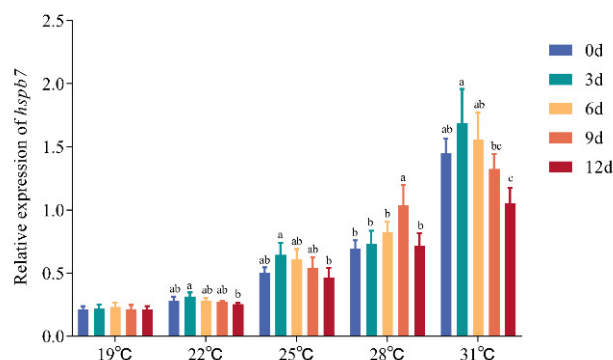


Figure 6. Changes of *hspb7* gene expression in abdominal tissue of roughskin sculpin under different temperature stress

the largest expression difference occurring after nine days of heat stress.

hspb7 gene expression was significantly higher in the 25 °C group than in the 19 °C and 22 °C groups from 0 to 12 days ($P < 0.05$). After 0, 3, 6, 9, and 12 days of heat stress, the differences in *hspb7* gene expression between the 25 °C and 19 °C groups were 2.4-fold, 3.0-fold, 2.6-fold, 2.5-fold, and 2.2-fold, respectively. The overall growth multiple initially increased and then decreased over time, with the largest expression difference occurring after three days of heat stress. *hspb7* gene expression did not differ significantly between the 19 °C and 22 °C treatment groups, but *hspb7* expression continued to increase.

There was no significant difference in *hspb7* gene expression between the experimental time points in the 19 °C group ($P > 0.05$; [Figure 6](#)). In the 22 °C group, there was no significant difference in *hspb7* gene expression at other time points, except that there was a significant difference in *hspb7* gene expression between 3 and 12 days after heat stress ($P < 0.05$). In the 25 °C group, the *hspb7* gene expression pattern was consistent with that in the 22 °C group. *hspb7* gene expression increased significantly after nine days of heat stress in the 28 °C group. In the 31 °C group, *hspb7* gene expression after three days of heat stress significantly differed from that after 9 and 12 days of heat stress ($P < 0.05$).

HSPB11 GENE EXPRESSION IN THE WHOLE FISH TISSUE

After 0–12 days of heat stress, *hspb11* gene expression significantly differed in the 31 °C group from other temperature groups, except for no significant difference between the 31 °C and 28 °C groups after 0 days of heat stress. *hspb11* gene expression in the 31 °C group was more than three times higher than that in the 19 °C group, with the largest difference occurring after three days of heat stress. The overall growth rate initially increases and then decreases over time.

The *hspb11* gene expression did not differ significantly in the 28 °C group than that in the 25 °C group after 12 days of heat stress ($P > 0.05$). However, *hspb11* gene expression at 0, 3, 6, and 9 days was significantly higher in the 28 °C

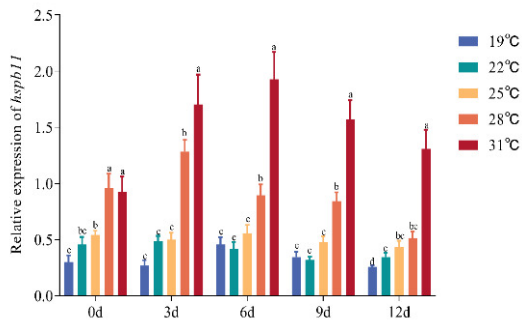


Figure 7. Temporal changes of *hspb11* gene expression in abdominal tissue of roughskin sculpin under different temperature stress

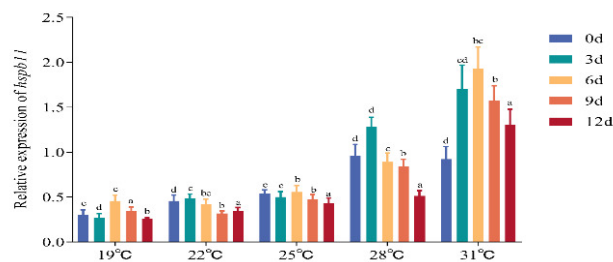


Figure 8. Changes of *hspb11* gene expression in abdominal tissue of roughskin sculpin under different temperature stress

group than in the 25 °C group ($P < 0.05$). After 0 to 12 days of heat stress, the *hspb11* gene expression was significantly higher in the 28 °C group than in the 19 and 22 °C groups ($P < 0.05$). After 0, 3, 6, 9, and 12 days of heat stress, the ratio of *hspb11* gene expression in 28 and 19 °C groups were 3.2-fold, 4.8-fold, 2.0-fold, 2.4-fold, and 2.0-fold, respectively, with the greatest expression difference occurring after three days of heat stress.

hspb11 gene expression was significantly higher in the 25 °C group than in the 19 °C treatment group ($P < 0.05$), except for heat stress at 0 and 12 days. The *hspb11* gene expression between the 19 and 22 °C groups differed significantly only after 12 days of heat stress ($P < 0.05$).

hspb11 gene expression was significantly different in the 31 °C group and was more than 5-fold higher in the 31 °C group than in the 19 °C group (Figure 8). The *hspb11* gene expression increased by 10.3-fold and 11.1-fold after heat stress for 3 and 12 days, respectively. In the 28 °C group, there was a significant difference in *hspb11* gene expression after different days of heat stress, with the expression level reaching its peak after three days of heat stress, indicating an expression pattern of initially increasing and then decreasing.

DISCUSSION

SURVIVAL RATE OF ROUGHSKIN SCULPIN UNDER DIFFERENT TEMPERATURE HEAT STRESSES

The water temperature is an important environmental factor affecting fish growth and development.^{14,15} Fish are usually a variable temperature animal that cannot maintain a constant body temperature when the ambient temperature changes.¹⁶ When the water temperature exceeded the optimal water temperature for survival, morbidity, and mortality,¹⁷ the survival rate of roughskin sculpin was investigated under different heat stress temperatures. The study found that some roughskin sculpins became darker, and their feeding decreased after five days of stress at 28 °C. It can be seen that 28 °C is a dangerous water temperature for their culture, and the roughskin sculpin has an obvious stress response at this temperature. After three days of 31 °C stress, the roughskin sculpin began to die, and almost all the roughskin sculpin died after five days of 33 °C stress. Therefore, the lethal temperature of roughskin sculpin was 31 °C, and the total lethal temperature was 33 °C.

MICROSCOPIC PATHOLOGICAL EFFECTS OF HEAT STRESS ON THE GILLS

The gills are the most important respiratory organs in fish and are mainly involved in osmotic pressure regulation, respiratory metabolism, and other functions.¹⁸ To obtain sufficient dissolved oxygen in the water, the gills, gill filaments, and gill lamellae of fish are completely open, and dense capillaries are distributed on the gill filaments.^{19,20} During gas exchange, the dissolved oxygen absorbed by the gill lamellae is transported to the whole body via blood circulation, and then the carbon dioxide produced in the body is transported to the outside through the gill lamellae.²¹ In this study, heat stress caused severe damage to the gills of roughskin sculpin. At 28 °C, many adjacent gill lamellae were fused, severely deformed, and curled in disorder, indicating that temperature stress caused direct damage to the roughskin sculpin's gill tissue.

When the heat stress temperature reached 31 °C, the respiratory epithelial cells of the gill lamellae swelled and proliferated, and the thickened gill lamellae, after proliferation, caused the gill filaments to 'stick-like,' indicating that heat stress caused direct damage to the roughskin sculpin's gills as well as damage to the gill defense response. As the heat stress temperature increased, the gill filaments and lamellae fell off, aggravating direct damage. The swelling and hyperplasia of the gill lamellae inhibited the respiration of the gills of the roughskin sculpin, and the ulceration and shedding of gill filaments and gill lamellae greatly reduced the respiratory function of the gills, thereby inhibiting the ability of the sculpin to cope with heat stress and reduce the survival ability of the roughskin sculpin. After 10 days of continuous heat stress at 31 °C, the gills of Hoven's carp showed similar histological lesions as in this study, including gill epithelial hyperplasia and hypertrophy, hemorrhage and congestion, and a large amount of

mucus.²² Similarly, *Labeo rohita* showed edema, hyperplasia, and necrosis of the gill filaments after 30 days of stress at 40 °C.²³

EFFECTS OF HEAT STRESS ON THE HEAT STRESS PROTEIN GENE EXPRESSION IN WHOLE BODY TISSUES

Heat shock proteins (HSPs) are synthesized by heat shock when fish are exposed to high-temperature stress.²⁴ Studies have shown that the increase in cell survival rate and anti-apoptotic ability under heat stress is related to HSP expression, and *hspb1* plays an anti-apoptotic role at the mitochondrial level.^{25,26} When water temperature changes, heat shock proteins can mediate the formation of protein-specific structures, thereby improving the protein's ability to respond to environmental changes.²⁷ In this study, we explored the expression patterns of HSP genes (*hspb1*, *hspb7*, and *hspb11*) in whole fish tissues of roughskin sculpin in response to heat stress. *hspb1* and *hspb7* gene expression did not change significantly within 12 days of heat stress in the 19 °C and 22 °C groups, but *hspb11* gene expression changed significantly, but there was no obvious trend. This indicated that under heat stress at 19 °C and 22 °C, the *hspb1* and *hspb7* genes in the sculpin had no obvious response to heat stress, while the *hspb11* gene had an obvious response to heat stress. In the 25 °C group, *hspb1* gene expression did not change significantly, whereas *hspb7* and *hspb11* gene expression initially increased and then decreased, indicating that the *hspb7* and *hspb11* genes in roughskin sculpin were more significant in response to heat stress at 25 °C. In the 28 °C group, the *hspb1* gene expression level increased significantly after three days of stress, while the *hspb7* and *hspb11* gene expression levels showed significant changes after nine days of stress, indicating that the *hspb1*, *hspb7*, and *hspb11* genes in roughskin sculpin responded more significantly under heat stress at 28 °C. The response mechanisms and response times of the three were different. In the 31 °C group, *hspb1*, *hspb7*, and *hspb11* gene expression levels initially increased and then decreased. The *hspb1* and *hspb11* gene expression levels increased significantly after three and nine days of heat stress, respectively, while the *hspb7* and *hspb11* gene expression levels decreased significantly after 12 days of heat stress. This indicates that the *hspb1*, *hspb7*, and *hspb11* genes in the roughskin sculpin were significant under heat stress at 31 °C and increased with heat stress, but the response mechanism and time of the genes remained different.

CONCLUSIONS

Heat stress seriously affects the respiratory function of the gills and threatens normal life activities. The stress response was significant at a water temperature of 28 °C. The roughskin sculpin died of heat stress at a water temperature of 31 °C. At this temperature, the proliferation of ep-

ithelial cells in the gills and other tissue damage jointly threaten the survival of the roughskin sculpin. Roughskin sculpin could not survive at a water temperature of 33 °C. HSP genes (*hspb1*, *hspb7*, and *hspb11*) play important roles in the response to heat stress. This study provides certain reference data for understanding the regulatory mechanism of heat stress resistance in roughskin sculpin and provides new ideas for studying the prevention measures of heat stress resistance.

ACKNOWLEDGMENTS

This work was supported by the Central Public-Interest Scientific Institution Basal Research Fund, CAFS (2023TD41), the Key R&D Program of Hebei Province, China (21326307D), and the National Marine Genetic Resource Center.

AUTHORS' CONTRIBUTION

Writing – original draft: Zitong Liu (Lead). Supervision: Lize San (Lead). Formal Analysis: Zhongwei He (Lead). Investigation: Zhongwei He (Lead). Funding acquisition: Yufeng Liu (Lead). Methodology: Tian Han (Lead). Data curation: Chunguang Gong (Lead). Resources: Jiangong Ren (Lead). Project administration: Yuqin Ren (Lead). Writing – review & editing: Jilun Hou (Lead).

COMPETING OF INTEREST – COPE

No competing interests were disclosed.

ETHICAL CONDUCT APPROVAL – IACUC

This study was performed in accordance with the Guidelines for Care and Use of Laboratory Animals of the Chinese Association for Laboratory Animal Sciences (No. 2011-2). The study protocols were approved by the animal care and use committee of Beidaihe Central Experiment Station, Chinese Academy of Fishery Sciences.

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

All are available upon reasonable request.

Submitted: March 14, 2024 CST. Accepted: April 09, 2024 CST.

Published: January 06, 2025 CST.



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