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Original Research Articles

Effects of Thermal Shock from Coastal Nuclear Power Plant Discharges on the Survival of Four Fish Species Under Variable Temperature Rise

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In this study, we simulated water temperature changes under variable frequency temperature rise conditions caused by coastal nuclear power plant discharges and conducted thermal shock tests on four fish species: *Trachinotus ovatus*, *Nibea albiflora*, *Larimichthys crocea*, and *Acanthopagrus schlegelii* at acclimated water temperatures of 25.0°C and 27.5°C during the summer. The effects of these temperature variations on the thermal shock response of the four fish species were analyzed. The results indicated that at 25.0°C, the mortality rate of *N. albiflora* exhibited an overall upward trend with increasing temperature and duration frequency, with an average mortality rate ranging from 10±3.3% to 38.9±3.3%. For *L. crocea*, mortality was observed only in the 8.5°C-100% duration probability group, while other groups had a 0% mortality rate. At 27.5°C, *A. schlegelii* showed an average mortality rate of 10±3.3% at an 8.5°C-100% duration probability, with all other groups showing 100% survival. The average mortality rate of *T. ovatus* at 8.5°C-100% was 6.7±3.3%, with no mortality in the other treatment groups. The expression level of the *hsp70* gene in the liver of *N. albiflora* increased with higher temperature rise amplitudes and longer frequency conversion durations. Similarly, the *hsp70* gene expression in *L. crocea* and *A. schlegelii* increased with rising temperatures, though there were no significant differences among groups with varying frequency conversion times. In contrast, the *hsp70* gene expression in *T. ovatus* remained relatively stable across temperature rise treatments, showing no significant differences with varying frequency conversion durations. The heat stress tolerance ranking among the four fish species was determined to be *L. crocea* > *N. albiflora* > *T. ovatus* > *A. schlegelii*.

INTRODUCTION

Temperature is one of the most critical environmental factors for fish, influencing physiological and biochemical processes such as metabolism, immunity, and digestive enzyme activity.[1,2] Heat stress involves a range of non-specific physiological responses to thermal stimuli at elevated temperatures.[3] Heat shock proteins (HSPs), also known as stress proteins, are a family of proteins expressed in response to various biotic and abiotic stressors.[4] Among them, *HSP70* is particularly significant, serving as a biomarker for evaluating heat stress responses in fish, and is one of the key physiological and biochemical indicators of thermal stress in fish.[5] Numerous studies have demonstrated that under heat stress, the expression of *HSP70* in tissues such as the liver, heart, gills, intestines, and brain of various fish species shows either an upward or downward trend with increasing external temperatures, indicating tissue specificity.[6]

The temperature adaptability of different fish species determines their spatiotemporal distribution. *T. ovatus* (*Trachinotus ovatus*) is a warm-water fish primarily found in tropical and temperate regions, including Chinese waters.[7] *N. albiflora* (*Nibea albiflora*) is a demersal fish inhabiting

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shallow coastal waters, mainly in China's temperate and subtropical coastal areas.[8] *L. crocea* (*Larimichthys crocea*) is a warm-temperate coastal migratory fish, with fishing grounds concentrated in the coastal areas of Jiangsu, Zhejiang, and Fujian.[9] *A. schlegelii* (*Acanthopagrus schlegelii*) is a widely distributed eurythermal and euryhaline demersal fish found in the Yellow Sea, Bohai Sea, East China Sea, and South China Sea.[10] These four species are important economic fish in China and hold significant value for coastal aquaculture.

With the rapid development of the nuclear power industry, the natural marine environment in coastal areas is increasingly disrupted. Thermal discharges from nuclear power plants raise water temperatures in receiving areas, altering the physicochemical properties of the water and potentially impacting the reproduction and growth of fish to varying extents.[11] Recent studies have investigated the impact of nuclear power plant thermal discharges on the thermal tolerance of economically important fish species in China under different ranges and rates of temperature increase.[12,13] However, research on the response of fish to thermal stress under varying frequency temperature increases remains limited, highlighting the need for further investigation.

This study focuses on the regional and economic significance of the selected fish species: *N. albiflora*, *L. crocea*, *A. schlegelii*, and *T. ovatus*. Thermal shock experiments were conducted under indoor simulated variable frequency conditions to mimic the temperature increases associated with nuclear power plant discharges. This research compares the thermal shock responses of these four fish species under different frequencies of temperature rise. The findings provide essential scientific data and technical support for assessing the impact of thermal discharges from nuclear power plants on marine biological resources.

MATERIALS AND METHODS

EXPERIMENTAL MATERIALS

A. schlegelii and *T. ovatus* were obtained from the Shenzhen Experimental Base of the South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences. The average body length and weight of *A. schlegelii* were 5.65±0.46 cm and 4.57±0.48 g, respectively. For *T. ovatus*, the average body length and weight were 6.57±0.19 cm and 8.93±0.62 g, respectively. *N. albiflora* and *L. crocea* were sourced from the Zhejiang Ninghai Base of the East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences. The average body length and weight of *N. albiflora* were 5.79±0.38 cm and 3.34±0.63 g, respectively, while those of *L. crocea* were 7.05±0.25 cm and 5.17±0.61 g, respectively.

The experimental water was natural seawater collected near the experimental base and was treated through sedimentation, sand filtration, and disinfection. The experiments were conducted from May to June 2022.

EXPERIMENTAL METHODS

ACCLIMATION

The four fish species were initially housed temporarily in culture tanks. Due to the high natural water temperatures in summer and the potential negative impact of thermal discharges on aquatic life, two acclimation temperatures were set based on the summer surface seawater temperatures in natural sea areas outside the mixing zones of coastal nuclear power plants in Guangdong and Zhejiang provinces: 27.5°C for *A. schlegelii* and *T. ovatus*, and 25.0°C for *N. albiflora* and *L. crocea*. The water temperature was adjusted to the respective acclimation temperatures by using a temperature control device. Acclimation lasted for seven days, during which the fish were fed artificial feed twice daily (at 09:00 and 17:00). Residual feed and feces were removed after 30 minutes, and one-third of the seawater was replaced. Continuous aeration was maintained to keep dissolved oxygen levels above 6.0 $mg{\cdot}L^{-1}$, with natural light and a photoperiod of 12 hours light and 12 hours dark. Healthy, active fish of similar size were selected for the experiments post-acclimation.

THERMAL SHOCK TREATMENT

Variable frequency temperature rise is defined as the proportion of time a specific temperature rise is maintained within a 24-hour period. Based on the thermal discharge conditions of two nuclear power plants and the hydrological characteristics of the sea area, temperature rise amplitudes of 1.0°C, 2.0°C, 4.0°C, and 8.5°C were set, with each temperature rise maintained for 25%, 50%, and 100% of 24 hours (i.e., 6 hours, 12 hours, and 24 hours). Thermal shock experiments were conducted in 100-liter glass tanks containing 80 liters of water. The tanks were submerged in culture pools with water levels the same as those in the glass tanks, and temperature was controlled using a combination of cooling and heating devices (±0.5°C). After reaching the predetermined temperature rises, 30 fish were placed in each tank. Heating was stopped after the specified durations (6 hours, 12 hours, and 24 hours). The total duration of experiment was 24 hours. Control groups without temperature rise were established for comparison, with three replicates for each temperature rise and control group. Fish were not fed during the experiments, and dissolved oxygen levels were maintained above 6.0 $mg{\cdot}L^{-1}$. Water temperature was monitored using a multiparameter water quality analyzer (YSI 650 MDS), and fish survival, stress responses, and mortality were recorded.

RNA EXTRACTION AND CDNA SYNTHESIS

After the experiments, liver tissues from three randomly selected fish in each experimental group were rapidly frozen in liquid nitrogen and stored at -80°C. Total RNA was extracted from various tissues using the Animal Total RNA Rapid Extraction Kit (Sangon Biotech, Shanghai), and RNA concentrations were measured with a UV spectrophotometer. cDNA synthesis was performed using a reverse

Table 1. The *hsp70* and internal reference gene primer sequences of four marine fish

Test organism	*Gene name*	*Primer sequence*
N. albiflora	*HSP70*	F 5'-TGAACAAGAGCATCAACCCAGAT-3' R 5'-TAGTCAACTTCCTCAATAGTGG-3'
	β-actin	F 5'-CCTCCCTGGAGAAGAGCTATGAG-3' R 5'-CGCACTTCATGATGCTGTTGTAG-3'
T. ovatus	*HSP70*	F 5'-AAGAAGGACATCAGCCAGAA-3' R 5'-AGAAGTCAGTGCCCTCAAATA-3'
	β-actin	F 5'-GTCATGTGGATCAGCAAGCAGGA-3' R 5'-CGCCCGAGTGTGTATGAGAAATG-3'
L. crocea	*HSP70*	F 5'-GGAAAGTTCGAGCTGACGGG-3' R 5'-CAGACGGCCTTTATCGTTGGTG-3'
	β-actin	F 5'-ACCCAGATCATGTTCGAGACC-3' R 5'-ATGAGGTAGTCTGTGAGGTCG-3'
A. schlegelii	*HSP70*	F 5'-CTCTGCGGAGATCCTTCAACA-3' R 5'-ACAAGAATAGGTGGTGCCCAG-3'
	β-actin	F 5'-CTGTCCCTGTATGCCTCTGGTC-3' R 5'-CTTGATGTCACGCACGATTTCC-3'

transcription kit (Sangon Biotech, Shanghai) according to the manufacturer's instructions, and the synthesized cDNA was stored at -20°C.

Primers for *HSP70* and internal reference genes were designed using Primer 5.0 software, based on sequences available in the NCBI database and transcriptome sequencing results. (Table 1) These primers were verified using the aforementioned reverse transcription template and synthesized by Sangon Biotech (Shanghai). Real-time PCR amplification and data analysis were conducted using the Real Time PCR EasyTM-SYBR Green I kit (Foregene, Chengdu) on a QuantStudio™ 7 Flex Real-Time PCR system.

DATA ANALYSIS

Statistical results are presented as the mean ± standard deviation (Mean ± SD). Data was analyzed using IBM SPSS Statistics 22.0 (IBM Inc., USA). Normality and homogeneity of variance tests were conducted on the experimental data. One-way ANOVA was used to analyze differences among groups, and Duncan's test was employed for multiple comparisons when significant differences were found. The significance level was set at 0.05.

RESULTS

EFFECTS OF DIFFERENT DURATION FREQUENCIES OF TEMPERATURE RISE ON THE MORTALITY RATES OF FOUR FISH SPECIES

The effects of different duration frequencies of temperature rise on the mortality rates of *N. albiflora*, *L. crocea*, *A. schlegelii*, and *T. ovatus* are presented in Table 2. At the baseline temperature of 25°C, no mortality was observed in the control group or the 1.0°C-25% duration frequency temperature rise group for *N. albiflora*. However, all the other experimental groups for *N. albiflora* exhibited varying degrees of mortality. As the experimental temperature and duration frequency increased, the overall mortality rate of *N. albiflora* presented an upward trend, with the highest mortality rate of 38.9±3.3% observed in the 8.5°C-100% duration frequency temperature rise group.

For *L. crocea*, no mortality was observed in the control group or in any temperature rise frequency groups, except for the 8.5°C-100% duration frequency temperature rise group, which had an average mortality rate of 10±3.3%.

At a baseline temperature of 27.5°C, *A. schlegelii* and *T. ovatus* showed a small amount of mortality in the 8.5°C-100% duration frequency temperature rise group, with average mortality rates of 10±3.3% and 6.7±3.3%, respectively. All the other temperature rise frequency groups and the control group had a 100% survival rate.

EFFECTS OF DIFFERENT DURATION FREQUENCIES OF TEMPERATURE RISE ON HSP70 GENE EXPRESSION IN LIVER TISSUES OF FOUR FISH SPECIES

EFFECTS ON HSP70 GENE EXPRESSION IN N. ALBIFLORA LIVER

At a baseline temperature of 25.0°C, *hsp70* gene expression in the liver of *N. albiflora* increased with rising temperatures. Specifically, the 2.0°C, 4.0°C, and 8.5°C temperature rise groups exhibited significantly higher *hsp70* gene expression levels compared to the control group ($p<0.05$) (Figure 1). Additionally, at an 8.5°C temperature rise, the *hsp70* gene expression level at a 100% duration frequency was significantly higher compared to the 25% and 50% duration frequencies, nearly doubling the levels observed in the latter.

EFFECTS ON HSP70 GENE EXPRESSION IN L. CROCEA LIVER

At a baseline temperature of 25.0°C, *hsp70* gene expression in the liver of *L. crocea* increased with rising temperatures. The 4.0°C and 8.5°C temperature rise groups showed significantly higher *hsp70* gene expression levels compared to

Table 2. Mortality rate of four fish species under different variable frequency temperature rise conditions (%)

Experimental group	*N. albiflora*	*L. crocea*	*A. schlegelii*	*T. ovatus*
Control group	0	0	0	0
1.0°C-25%	0	0	0	0
1.0°C-50%	10±3.3	0	0	0
1.0°C-100%	10±3.3	0	0	0
2.0°C-25%	10±5.8	0	0	0
2.0°C-50%	10±3.3	0	0	0
2.0°C-100%	15.6±8.8	0	0	0
4.0°C-25%	10.0±6.7	0	0	0
4.0°C-50%	20.0±6.7	0	0	0
4.0°C-100%	30.0±3.8	0	0	0
8.5°C-25%	16.7±3.3	0	0	0
8.5°C-50%	30.0±3.3	0	0	0
8.5°C-100%	38.9±3.3	10.0±3.3	10.0±3.3	6.7±3.3

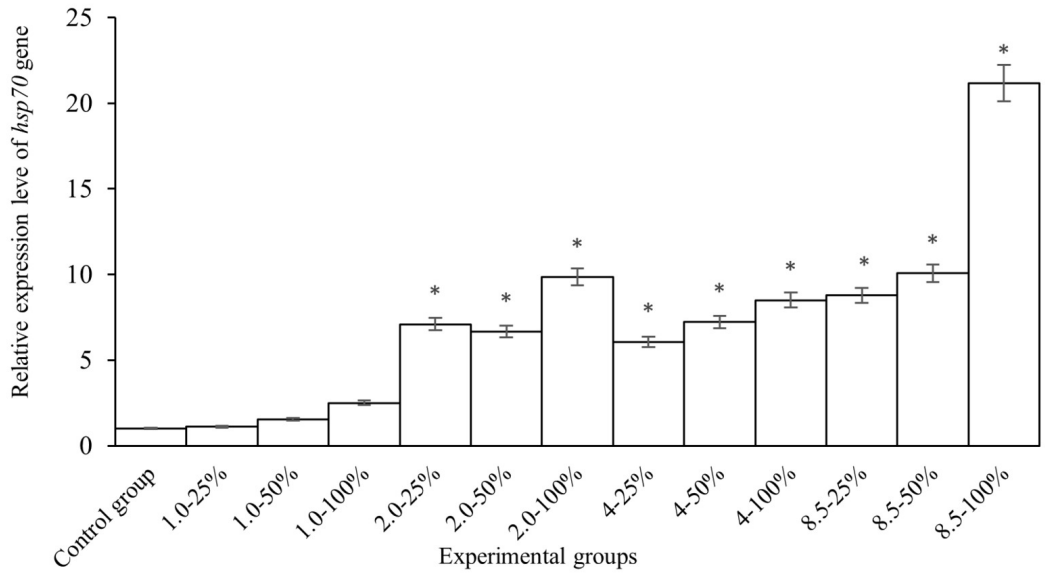

Fig. 1. Effect of different heating frequencies on the expression of *hsp70* gene in *N. albiflora*

Note:*indicates a significant difference compared to the control group, p<0.05,the same below

the control group (p<0.05) (Figure 2). However, under the same temperature rise, *hsp70* gene expression levels fluctuated with different duration frequencies, showing no significant differences between the groups (p>0.05) (Figure 2).

EFFECTS ON HSP70 GENE EXPRESSION IN A. SCHLEGELII LIVER

At a baseline temperature of 27.5°C, *hsp70* gene expression in the liver of *A. schlegelii* increased with rising temperatures across all experimental groups. However, only the 8.5°C temperature rise group showed a significant difference compared to the control group (p<0.05). Under the same temperature rise, *hsp70* gene expression levels remained relatively stable across different duration frequency groups (Figure 3).

EFFECTS ON HSP70 GENE EXPRESSION IN T. OVATUS LIVER

At a baseline temperature of 27.5°C, *hsp70* gene expression in the liver of *T. ovatus* remained stable across all temperature rise groups, showing no significant differences compared to the control group (p>0.05). The *hsp70* gene expression levels fluctuated across different duration frequency

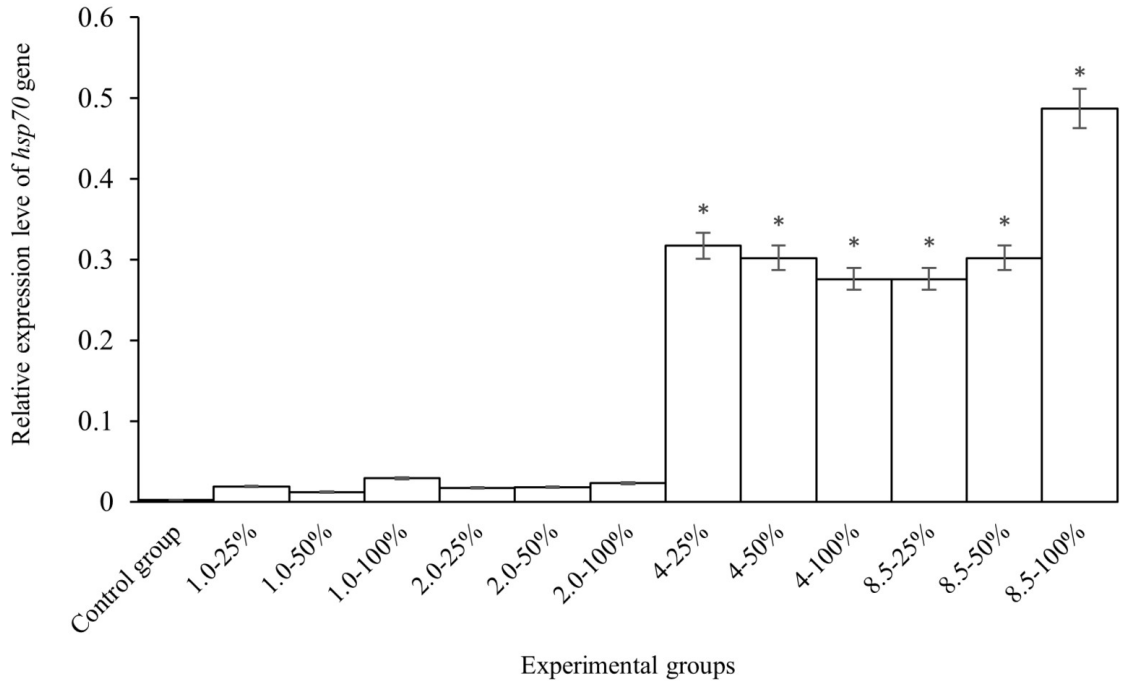

Fig. 2. Effect of different heating frequencies on the expression of *hsp70* gene in *L. crocea*

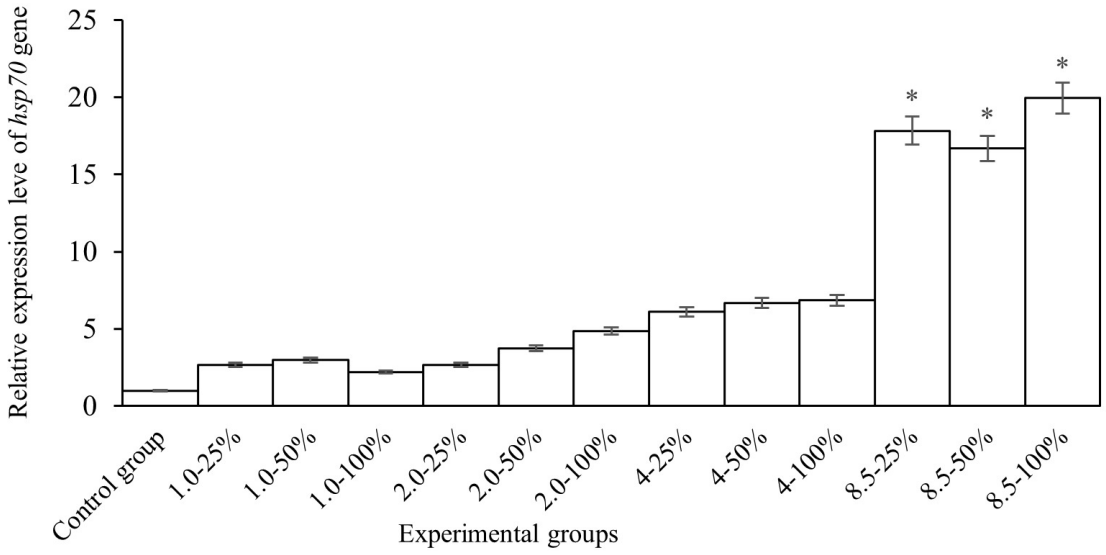

Fig. 3. Effect of different heating frequencies on the expression of *hsp70* gene in *A. schlegelii*

groups, but no significant differences were observed between them ($p>0.05$) (Figure 4).

DISCUSSION

EFFECTS OF TEMPERATURE RISE ON THERMAL SHOCK IN FOUR FISH SPECIES

The results (Table 3) indicate that during thermal shock experiments at a baseline temperature of 25.0°C, *N. albiflora*

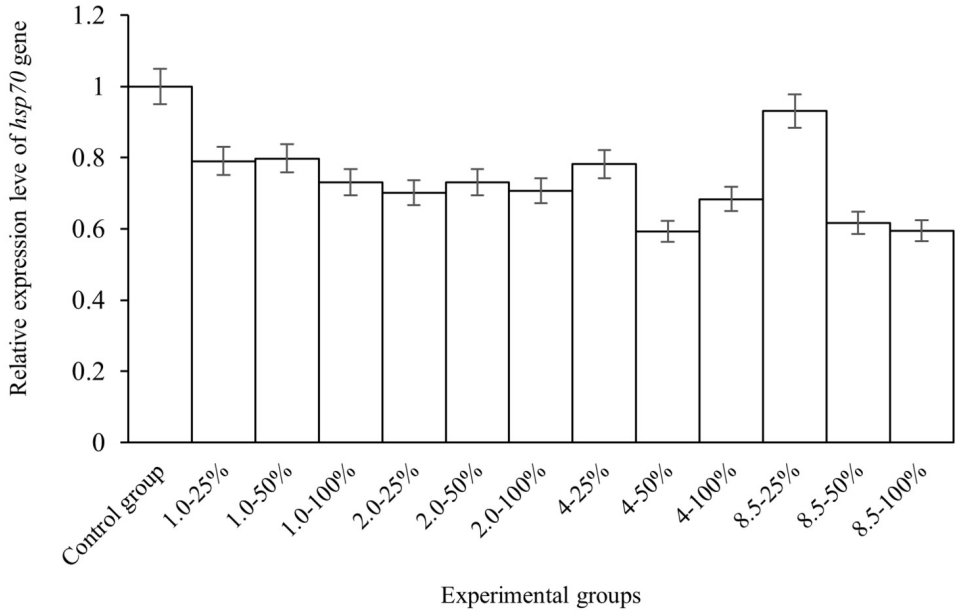

Fig. 4. Effect of different heating frequencies on the expression of hsp70 gene in *T. ovatus*

experienced varying degrees of mortality across the 1°C, 2°C, 4°C, and 8.5°C temperature rise groups, with higher mortality rates at increased temperatures. The 8.5°C temperature rise groups exhibited mortality rates ranging from 16.7% to 38.9%, significantly higher than the other groups. In contrast, *L. crocea* showed only minor mortality in the 8.5°C-100% duration frequency group (around 10%) and no deaths in other conditions.

Fish can adapt to temperature changes within a suitable range through biochemical, metabolic, and physiological processes.[14] However, unsuitable temperatures have negative impacts on fish. The rise in temperature from 1°C to 2°C, 4°C, and 8.5°C likely affected the physiological functions of some *N. albiflora*s, leading to increased mortality. *L. crocea*, with a temperature tolerance range of 10-33°C,[15] only showed minor mortality at temperatures between 26°C and 33.5°C, indicating it had not exceeded its stress tolerance limit.

Thermal tolerance in marine organisms is influenced by environmental changes and determined by genetic traits and physiological functions.[16] The results show that *L. crocea* has higher thermal tolerance than *N. albiflora* under the same temperature rise conditions. At a baseline temperature of 27.5°C, *A. schlegelii* and *T. ovatus* experienced no mortality at 1°C, 2°C, and 4°C temperature rises. However, the 8.5°C-100% duration frequency group showed about 10% mortality for *A. schlegelii* and 6.7% for *T. ovatus*.

A. schlegelii, a eurythermal and euryhaline species, has a thermal limit of 34°C.[10] The test temperature of 36.0°C (8.5°C temperature rise group) likely exceeded its thermal tolerance, causing stress reactions and mortality. In contrast, *T. ovatus*, a warm-water species,[17] showed stronger thermal tolerance.

Thermal discharge varies dynamically with temperature rise duration and water flow, affecting fish thermal tolerance.[18] Under conditions of 1°C and 2°C temperature rise, the high-temperature duration had no significant correlation with the mortality rates of the four fish species. However, under 4°C and 8.5°C temperature rise conditions, the mortality rate of *N. albiflora* increased with longer duration frequencies. Similarly, under 8.5°C temperature rise conditions, all four fish species exhibited varying degrees of mortality at 100% duration frequency.

Fish are poikilothermic animals. A suitable temperature rise range enables them to adjust and adapt to environmental changes. However, near their thermal tolerance limit, fish experience high-temperature stress, affecting their immune systems and resistance.[19] Prolonged exposure to high temperatures without adequate time for self-adjustment leads to activity disorders and mortality.

EFFECTS OF TEMPERATURE RISE ON HSP70 GENE EXPRESSION IN FOUR FISH SPECIES

The heat shock response pathway regulates the expression of *hsp70* genes in fish. *Hsp7* binds to denatured proteins, assisting in their folding and assembly, and its expression significantly increases under heat stress, enhancing cellular resistance to external stimuli and improving survival rates.[20] The results indicate that *hsp70* gene expression in *N. albiflora* liver significantly increased under temperature rise conditions of 2°C, 4°C, and 8.5°C ($p<0.05$). In *L. crocea*, *hsp70* gene expression significantly increased under temperature rise conditions of 4°C and 8.5°C ($p<0.05$).

These findings suggest a protective heat stress response in *N. albiflora* and *L. crocea*, where rapid synthesis of *hsp70* proteins enhances tolerance to heat stress.[21] Similarly, the

8.5°C temperature rise group significantly increased *hsp70* gene expression in *A. schlegelii* liver. These changes in *hsp70* gene expression are consistent with observations in other fish species, such as Carassius auratus,[22] I,[23] and *Centropristis striata*,[24] under acute heat stress, where *hsp70* mRNA levels significantly rise.

Fish are sensitive to heat stress, triggering physiological adjustments such as *hsp70* gene activation to maintain homeostasis.[25] *T. ovatus*, a warm-water fish with a temperature range of 16-36°C,[26] showed no significant increase in *hsp70* gene expression under the test conditions, likely because the temperature did not exceed its tolerance range.

Additionally, the study observed that in *L. crocea* and *A. schlegelii*, *hsp70* gene expression showed no significant differences under different duration frequencies at 4°C and 8.5°C temperature rises. However, in *N. albiflora*, the rise in *hsp70* gene expression at 8.5°C temperature was significantly higher at 100% duration frequency compared to 25% and 50%, indicating increased thermal stress damage.

CONCLUSIONS

Mortality rates of *N. albiflora* increased with higher temperature rises, longer duration, and higher frequencies. *L. crocea*, *A. schlegelii*, and *T. ovatus* only exhibited minor mortality at the 8.5°C-100% duration frequency.

The *hsp70* gene expression in the liver of *N. albiflora* significantly increased under temperature rise conditions of 2°C, 4°C, and 8.5°C. In *L. crocea*, significant increases in *hsp70* gene expression were observed under temperatures of 4°C and 8.5°C. In *A. schlegelii*, a significant increase was noted under the 8.5°C temperature rise condition. *T. ovatus* showed no significant increase in *hsp70* gene expression under test conditions.

L. crocea exhibited higher thermal stress tolerance than *N. albiflora*, and *T. ovatus* showed higher tolerance than *A. schlegelii*.

AUTHORS' CONTRIBUTION

Conceptualization: Pengcheng Sheng (Lead). Formal Analysis: Pengcheng Sheng (Lead). Investigation: Pengcheng Sheng (Lead). Methodology: Jiaying Cai (Lead), Chenshan Shao (Supporting). Writing – original draft: Jiaying Cai (Lead), Xucheng Nie (Supporting). Writing – review & editing: Jiaying Cai (Supporting), Chenshan Shao (Lead). Resources: Yebing Yu (Supporting), Jiacheng Jiang (Lead), Zhanyu Sha (Supporting). Supervision: Mei Jiang (Supporting), Lei Li (Lead).

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

All are available upon reasonable request.

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REFERENCES

1. Liu M, Zhou Yl, Guo XF, et al. Comparative transcriptomes and metabolomes reveal different tolerance mechanisms to cold stress in two different catfish species. *Aquaculture*. 2022;560:738543. doi:10.1016/j.aquaculture.2022.738543

2. Gooseff MN, Strzepek K, Chapra SC. Modeling the potential effects of climate change on water temperature downstream of a shallow reservoir, lower madison river, MT. *Climatic Change*. 2005;68(3):331-353. doi:10.1007/s10584-005-9076-0

3. Rajaguru S. Thermal resistance time of estuarine fishes Etroplus suratensis and Therapon jarbua. *Journal of Thermal Biology*. 2002;27(2):121-124. doi:10.1016/S0306-4565(01)00025-0

4. da Silva CRB, Riginos C, Wilson RS. An intertidal fish shows thermal acclimation despite living in a rapidly fluctuating environment. *Journal of Comparative Physiology B*. 2019;189(3):385-398. doi:10.1007/s00360-019-01212-0

5. Logan LH, Gupta RS, Ando A, Suski C, Stillwell AS. Quantifying tradeoffs between electricity generation and fish populations via population habitat duration curves. *Ecological Modelling*. 2021;440:109373. doi:10.1016/j.ecolmodel.2020.109373

6. Shi K, Dong S, Zhou Y, et al. Comparative Evaluation of Toleration to Heating and Hypoxia of Three Kinds of Salmonids. *Journal of Ocean University of China*. 2018;17(6):1465-1472. doi:10.1007/s11802-018-3673-9

7. Kumar N, Thorat ST, Gite A, Patole PB. Selenium nanoparticles and omega-3 fatty acid enhanced thermal tolerance in fish against arsenic and high temperature. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 2022;261:109447. doi:10.1016/j.cbpc.2022.109447

8. Han M, Luo M, Yang R, Qin JG, Ma Z. Impact of temperature on survival and spinal development of *T. ovatus Trachinotus ovatus* (Linnaeus 1758). *Aquaculture Reports*. 2020;18:100556. doi:10.1016/j.aqrep.2020.100556

9. Beitinger TL, Bennett WA, McCauley RW. Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environmental Biology of Fishes*. 2000;58(3):237-275. doi:10.1023/A:1007676325825

10. Dominguez M, Takemura A, Tsuchiya M, Nakamura S. Impact of different environmental factors on the circulating immunoglobulin levels in the Nile tilapia, Oreochromis niloticus. *Aquaculture*. 2004;241(1):491-500. doi:10.1016/j.aquaculture.2004.06.027

11. Ji L, Jiang K, Liu M, et al. Low temperature stress on the hematological parameters and HSP gene expression in the turbot Scophthalmus maximus. *Chinese Journal of Oceanology and Limnology*. 2016;34(3):430-440. doi:10.1007/s00343-016-4367-z

12. Jia Y, Chen X, Wang Z, Meng Z, Huang B, Guan C. Physiological response of juvenile turbot (Scophthalmus maximus. L) during hyperthermal stress. *Aquaculture*. 2020;529:735645. doi:10.1016/j.aquaculture.2020.735645

13. Westwood JT, Clos J, Wu C. Stress-induced oligomerization and chromosomal relocalization of heat-shock factor. *Nature*. 1991;353(6347):822-827. doi:10.1038/353822a0

14. Zhou CW, Hu XW, Lei L, et al. Effects of Heat Stress on Biochemical Indices and HSP70 mRNA Expression in Gibel Carp (Carassius auratus gibelio). 2018;39(6):65-71. doi:10.19663/j.issn2095-9869.20171012001

15. Guo ZY, Jiao CZ, Xiang JH. Heat-shock protein 70 expression in shrimpFenneropenaeus chinensis during thermal and immune-challenged stress. *Chinese Journal of Oceanology and Limnology*. 2004;22(4):386-391. doi:10.1007/BF02843633

16. Shahi N, Ardó L, Fazekas G, et al. Immunogene expression in head kidney and spleen of common carp (Cyprinus carpio L.) following thermal stress and challenge with Gram-negative bacterium, Aeromonas hydrophila. *Aquaculture International*. 2018;26(3):727-741. doi:10.1007/s10499-018-0250-6

17. Kır M, Sunar MC, Altındağ BCJJotb. Thermal tolerance and preferred temperature range of juvenile meagre acclimated to four temperatures. 2017;65:125-129. doi:10.1016/j.jtherbio.2017.02.018

18. Hu J, Wu KC, Ye L, Yu W. Effect of acute salinity stress on catalase of juvinile Amphiprion clarkii. *South China Fisheries Science*. 2015;11(6):73-78. doi:10.3969/j.issn.2095-0780.2015.06.010

19. Huong DTT, Tram CHT, Ha NTK, Gam LTH, Ishimatsu A, Phuong NT. Effects of carbon dioxide (CO2) at different temperatures on physiological parameters and growth in striped catfish (Pangasianodon hypophthalmus) juveniles. *Aquaculture*. 2021;534:736279. doi:10.1016/j.aquaculture.2020.736279

20. Reyes-Becerril M, Angulo-Valadez C, Macias ME, Angulo M, Ascencio-Valle F. Iron bioavailability in larvae yellow snapper (Lutjanus argentiventris): Cloning and expression analysis of ferritin-H. *Fish & Shellfish Immunology*. 2014;37(2):248-255. doi:10.1016/j.fsi.2014.02.011

21. Iwama GK, Thomas PT, Forsyth RB, Vijayan MM. Heat shock protein expression in fish. *Reviews in Fish Biology and Fisheries*. 1998;8(1):35-56. doi:10.1023/A:1008812500650

22. Long Z, Qin H, Huang Z, Xu A, Ye Y, Li Z. Effects of heat stress on physiological parameters, biochemical parameters and expression of heat stress protein gene in Lateolabrax maculatus. *Journal of Thermal Biology*. 2023;115:103606. doi:10.1016/j.jtherbio.2023.103606

23. Fu C, Zhou KY, Hu Y, Zhang YF, Fu SJ. The effects of the predictability of acclimatory temperature on the growth and thermal tolerance of juvenile Spinibarbus sinensis. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. 2024;295:111652. doi:10.1016/j.cbpa.2024.111652

24. Xiaojuan XU, Jiaer LI, Youjun OUJSCFS. Effects of salinity on embryonic development and early larvae in ovate pompano *Trachinotus ovatus*. Published online 2009. doi:10.3969/j.issn.1673-2227.2009.06.006

25. Wang XL, Li L, Jian YX, et al. Anesthesia effect of clove oil on juvenile Nibea albiflora. *Transactions of Oceanology and Limnology*. 2023;45(3):31-37. doi:10.13984/j.cnki.cn37-1141.2023.03.005

26. Westgaard JI, Fevolden SE. Atlantic cod (Gadus morhua L.) in inner and outer coastal zones of northern Norway display divergent genetic signature at non-neutral loci. *Fisheries Research*. 2007;85(3):306-315. doi:10.1016/j.fishres.2007.04.001