KJEE 56(4): 420-429 (2023)
https://doi.org/10.11614/KSL.2023.56.4.420
ISSN: 2288-1115 (Print), 2288-1123 (Online)

Original article

Fluctuation of Temperature Induces Pathogenicity of *Streptococcus iniae* and Changes of Immunology Related Genes of Korean Rockfish, *Sebastes schlegeli*

EunYoung Min (0000-0001-9151-6843), Seon-Myeong Jeong ((0009-0005-9657-9225)
Hyun-Ja Han (0000-0001-8982-9947) and Miyoung Cho* (0000-0001-7457-5200)

Pathology Division, National Institute of Fisheries Science, Busan 46083, Republic of Korea

Abstract This study was designed to examine the immune response in Korean rockfish during water temperature fluctuation and to elucidate the factors contributing to streptococcal pathogenesis in cultured Korean rockfish, *S. schlegeli*. We investigated cumulative mortality against *Streptococcus iniae* (FP5228 strain) infection in the exposed Korean rockfish (39.7 ± 5.8 g) to environmentally relevant temperature (Control, 23°C; High temperature, 28°C and 23°C and 28°C with 12 hours interval exchange, 23↔28°C) for 48 hours. Also, the expression of the mRNA related to the immune response genes (heat shock protein 70, interleukin-1β, lysozyme g-type and thioredoxin-like 1) were measured in spleen and head kidney by real-time PCR analysis in the exposed fish to thermal stress. In this study, the combined stress with bacterial challenge in fishes exposed to thermal stress lowered the survival rate than that of control (23°C). The cumulative mortality in the group of control, 28°C and 23↔28°C was 24%, 24% and 40% ($P < 0.05$), respectively. Also, thermal stress modulated the mRNA level of immune related genes; heat shock protein 70, interleukin-1β, lysozyme g-type and thioredoxin-like 1 in Korean rockfish. The present study indicates that a high and sudden water temperature change affect immune responses and reduce the disease resistance in Korean rockfish.

Key words: thermal stress, *Sebastes schlegeli*, the disease resistance, immune response

INTRODUCTION

The damage of aquaculture industry caused by natural disasters, such as frequent typhoon, global warming and abnormal change of weather, is on the rise (Harvell *et al.*, 2008; Lejeusne *et al.*, 2010). Recently, Lee *et al.* (2018) reported that the causes of mass mortality of cultured fish in the south coasts of Korea are harmful algal blooms (37.4%), high water temperature (31.0%), and low water temperature (26.5%) of total 1,367 outbreak cases from 1998 to 2016.

The importance of the water temperature tolerance of fish is increasing because of climate change caused by global warming. Because of the changes in aquatic environment such as climate change, the number of diseases in marine organisms is increasing. Particularly, in the summer of 2012, mass mortality occurred in black rockfish, *Sebastes schlegeli*, raised in floating fish cages along the coast of Gyeongsangnam-do in Korea. A rapid rise in water temperature was confirmed to be the cause of damage to 1,802,000 fishes (Lee *et al.*, 2013). The cause of this abnormal mortality being just

Manuscript received 7 October 2023, revised 27 December 2023, revision accepted 27 December 2023
* Corresponding author: Tel: +82-51-720-2731, Fax: +82-51-720-2498
E-mail: mycho69@korea.kr

the high temperature in summer, with no obvious indication of disease, is doubtful. Water temperature can cause significant changes in the physiology of ectothermic organisms and thus affect their immunity and sensitivity to pathogens. A pathogen itself is insufficient for a disease outbreak, without interference of environmental factors (Bowden, 2008). Also, Karvonen *et al.* (2010) demonstrated the effect of increasing water temperature on aquatic disease dynamics in northern Finland from 1986 to 2006. The experiment animals were exposed to a 'heat wave' of 28°C for 2 weeks, which resulted in long lasting immune disorders. The study suggested when such events occur naturally, they may result in animals becoming immune-compromised, which could facilitate the spread of infectious disease within these populations (Dittmar *et al.*, 2013).

Streptococcus sp. is a significant rockfish pathogen responsible for considerable losses in aquaculture in Korea (Choi *et al.*, 2010; Jung *et al.*, 2012). Particularly, *Streptococcus iniae* is a pathogen in fish, capable of causing invasive disease and outbreaks in global aquaculture farms (Agnew and Barnes, 2007). Also, it is known that the growth of the *S. iniae* depends on the temperature and the nutritional ingredients in the water (Jeun *et al.*, 2017).

This study was designed to examine the immune response of both *S. iniae* and black rockfish during water temperature fluctuation in order to elucidate the factors contributing to streptococcal pathogenesis in cultured black rockfish *S. schlegeli*.

MATERIALS AND METHODS

1. Fish acclimations and thermal stress

Black rockfish (*S. schlegeli*; body mass 39.7±5.8 g, body length 17.1±0.9 cm) were obtained from a commercial farm (Namhae, South Korea). Fish specimens were maintained in one-ton aerated PVC tanks in the 12/12 light cycles and were fed daily with a commercial diet at a rate of 3% body weight/day. Also, fish were held for 3 weeks in seawater at 23°C to ensure that all individuals were healthy and being fed, and also to reset the thermal history of the animals prior to initiating temperature acclimations. The water temperature was adjusted from ambient at a rate of ± 1°C/hour until the final temperature of 28°C was reached. One percent of the fish were randomly selected to perform bacterial isolation to ensure that they were *streptococcus iniae*-free.

Acclimated fish were divided into 3 experiment groups containing 30 fish per group, which were comprised of (1) group maintained at 23°C, (2) group exposed at 23°C and 28°C with 12 hours intervals exchange, and (3) group exposed at 28°C water temperature. The water temperature experiment groups used in this study was selected according to the "Real-Time Water Temperature Information" of Tongyeong on the south coast and Hyeopjae on Jeju Island in KOREA (the maximum water temperature 28.4°C, the minimum water temperature 21.2°C in July~August, 2018; the high-water temperature period of the year) (https://www.nifs.go.kr/ risa/main.risa). The experiment of thermal stress lasted for 48 hours in one-ton aerated PVC tanks. The water temperature was controlled by an electrical thermostat and checked twice daily.

2. Bacterial strain

The pathogen, *Streptococcus iniae* (FP5228 strain) used in this study was originally isolated from a diseased fish during streptococcosis outbreak in Jeju. The bacterial species was identified with standard biochemical assays and the identity was confirmed by PCR analysis targeting 16S rRNA, and the bacteria were stored at −70°C until use (Nho *et al.*, 2009). For experiments, bacteria were cultured at 37°C overnight using Tryptone soya agar (TSA; Difco, USA) with 2% NaCl(w/v). A single colony to an OD_{610nm} of 1.0 was washed with phosphate buffered saline (PBS), and suspended to 1×10^7 CFU in PBS.

3. Serum bactericidal activity assay

Fish were sampled at 3, 6, 12, 24 and 48 hours after thermal stress. Blood sample were collected within 35~40 s through the caudal vein of fish in 0.5 mL disposable heparinized syringes using a 30-gauge needle. The syringes were kept at room temperature for a while. Serum was obtained by centrifuging at 3,000 g for 15 min at 4°C and then kept in the refrigerator until use. Five fish from each treatment were sampled for serum.

Serum bactericidal activity was determined as described previously with some modifications (Rao *et al.*, 2005). Briefly, *S. iniae* bacterial culture was centrifuged and the pellet was washed and suspended in PBS. Optical density

Table 1. Primers sequence for RT-qPCR in this study.

Gene	Sequence (5′-3′)	Target size (bp)	Reference
Heat shock protein 70 gene (HSP70)	F: AGAGCCGGTGGAGAAAGC R: CCTCGTCTGGGTTGATGC	161	Mu *et al.* (2013)
Lysozyme gene, g-type (Lyg)	F: TGGGGACTGATGCAGGTTGA R: TGTACCACTGAGCTCTGGCA	283	This study
Interleukin-1 beta gene (IL1β)	F: ACCTGAACATGTCGACCTAC R: AGGTTGGATTGGCACCATTC	126	This study
Thioredoxin-like gene (Txnl1)	F: ATTCCACTGCGCTACGTCAA R: TACCGGAGTGCCTATGAATG	120	Modified from Kugapreethan *et al.* (2017)
Ribosomal protein L17 gene (Rpl17)	F: AGGCGACGCACCTACCG R: CCTCTGGTTTGGGGACGA	109	Liman *et al.* (2013)

(O.D) of the suspension was adjusted to 0.5 at 546 nm. This bacterial suspension was serially diluted (1 : 10) with PBS five times. Serum bactericidal activity was measured by incubating 2 μL of this diluted bacterial suspension with 20 μL of serum for 0 and 1 hour at 27°C. In the bacterial control group, PBS replaced the serum. After incubation, the number of viable bacteria was determined by counting the colonies grown on nutrient agar plate for 24 h at 27°C. Serum bacterial activity was calculated by the following method.

Bacterial activity = colony count after 1 hour incubation / colony count after 0 hour incubation

4. Immunological response analysis

Organs of fish were sampled at 3, 6, 12, 24 and 48 hours after thermal stress. Fish were deeply anesthetized using benzocaine, and the spleen and head kidney were removed and stored at −80°C until analysis. Total RNA was isolated from tissues pooled from five individual fish using QIAzol (Qiagen, USA), per the vendor's protocol, and further purified by the RNeasy Mini kit (Qiagen, USA). One microgram of total RNA was converted to the first-strand cDNA using an Omniscript RT kit (Qiagen, USA).

The gene-specific primers used for mRNA quantification by real-time qPCR were shown in Table 1. The genes of heat shock protein 70 (HSP 70), Interleukin-1 beta (IL1 β), Lysozyme g-type (Lyg) and Thioredoxin-like 1 (Txnl1) were selected in this study to evaluate immune response in fish, because it is well known that pathogen or thermal stress-derived genes are targets used by the host immune responses to control infection (Engelsma *et al.*, 2002; Shakoori *et al.*, 2019; Yu *et al.*, 2020). Ribosomal protein L17 gene, a housekeeping gene whose expression was found to be unaffected by the treatment in the present experiment, was used as an endogenous reference to normalize the template amount. The real-time qPCR analysis was performed using Applied Biosystems™ QuantStudio™ 5(Thermo Fisher Scientific, America) in a 20 μL reaction volume containing AccuPower GreenStar™ RT-qPCR Master Mix (Bioneer, Korea). The real-time qPCR temperature profile for all genes was 95°C for 5 min after 60°C for 15 min followed by 40 cycles of 15 s at 95°C, 30 s at 62°C. After the final cycle of PCR, the melting curves were systematically monitored (65°C temperature gradient at 0.5°C / 10 s from 65 to 95°C). During the detection, each sample was run in triplicate. PCR-grade water in place of the template served as the negative control. The $2^{-\triangle\triangle}$ method was used to analyze real-time qPCR data (Livak and Schmittgen, 2001). The mRNA levels of target genes were shown at the n-fold difference relative to the calibrator.

5. Cumulative mortality of fish affected by thermal stress

Black rockfish (n = 90) affected by previous thermal stress were employed in a challenge test to determine the host susceptibility to *S. iniae* infection. Bacteria used in this experiment had been passaged *in vivo* of fish at least one time prior to starting the artificial infection test. Fish were inoculated intra-peritoneally with either 3×10^5 CFU bacterial cells in PBS or PBS alone (control). The fish were divided into 3 groups containing 20 fish per group, which were comprised

of (1) *S. iniae* challenged group after exposure to 28°C, (2) *S. iniae* challenged group after exposure to 23°C and 28°C with 12-hour intervals exchange, and (3) *S. iniae* challenged group maintained at 23°C. The water temperature controlled by an electronic heater and checked twice daily during mortality test. After the challenge, clinical signs and mortalities were recorded for 2 weeks, while dead fish were removed rapidly to confirm whether *S. iniae* was the cause of mortality by using bacterial isolation. All experiments were performed in duplicate. No feed was provided during the challenge test period.

6. Statistical analysis

Statistical analyses were performed using the SPSS/PC+ statistical package (SPSS Inc, Chicago, IL, USA). Significant differences between the groups were identified using one-way analysis of variance (ANOVA) and Duncan's test for multiple comparisons. The significance level was set at $P<0.05$.

RESULTS AND DISCUSSION

Although various studies have predicted relationships between climate change and outbreaks of infectious diseases in fishery (Alborali, 2006; Marcos-Lopez *et al.*, 2010), the effects of a combination of marine environmental stressors in an intensive aquaculture remains elusive. Also, despite the fact that the daily water temperature changes in the summer of Gyeongnam area in Korea are more than 5~6°C, the effect of sudden temperature changes within a few hours has not been investigated. Therefore, in this study, it is examined the effect of thermal stress, including sudden temperature changes, on the immune response and disease resistance of black rockfish, *S. schlegeli*.

1. Serum bactericidal activity assay

The serum bactericidal activity assay carried out in this study assesses the complement-dependent bactericidal activity of antibodies in sera against bacterial isolations. This assay is the method of choice to evaluate the complement-mediated functional activity of both infection- and vaccine-induced antibodies (Boyd *et al.*, 2014). The effects of thermal stress on serum bactericidal activity of *S. iniae* are presented in Fig.

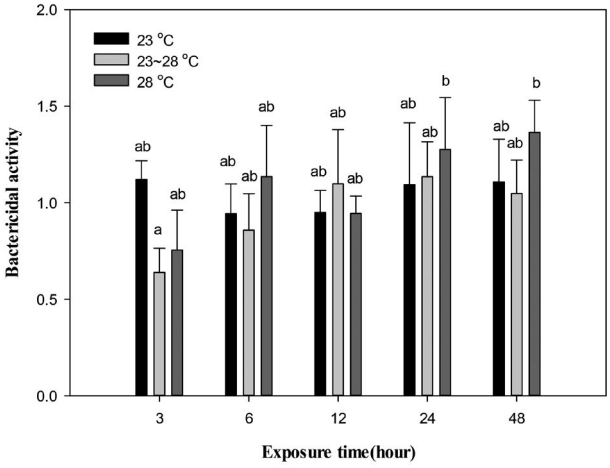

Fig. 1. Effects of thermal stress on serum bactericidal activity of *S. iniae*. The values represent the mean ± S.D of three replicates. Vertical bar denotes a standard error (n = 10). Values with different superscripts are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

1. No significant differences were observed in serum bactericidal activity in the group of control and 23↔28°C ($P>0.05$). After 24 h heat stress (28°C), serum bactericidal activities were significantly increased. Bactericidal activity in general has been found to be increased by the rise in temperature (Cooper and Haines, 1928), but *S. iniae* is reported to be able to withstand the bactericidal activity of the macrophages and can trigger apoptosis to facilitate its exit (Zlotkin *et al.*, 2003).

2. Cumulative mortality of fish affected by thermal stress

In Black rockfish, *S. schlegeli*, the sudden water temperature change (23↔28°C) increases the severity of streptococcosis in this study (Fig. 2). The fish in the control group (23°C) survived at 48% during the whole experimental period after *S. iniae*, bacterial challenge. The combined stress by bacterial challenge in fish exposed to higher (28°C) and fluctuation (23↔28°C) of water temperature lowered the survival rate than that of control group (23°C) after 6 days. After 14 days, the cumulative mortality in the group of control, 28°C and 23↔28°C is 24%, 24% and 40% ($P<0.05$), respectively (Fig. 2).

Generally, stressors could either directly affect fish mortality or indirectly by suppressing their immunity, allowing for disease outbreak following the invasion by bacteria.

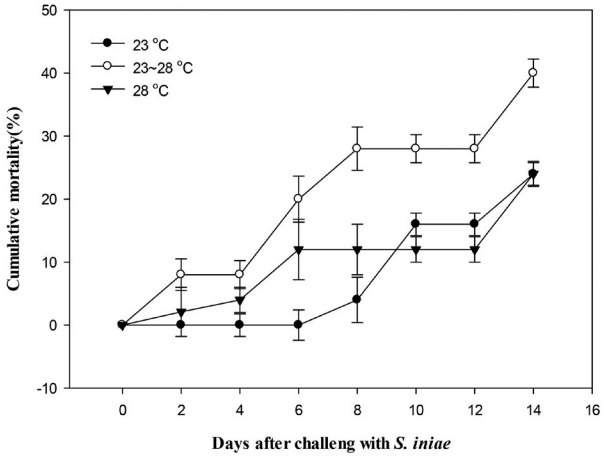

Fig. 2. Accumulated mortalities of Korean rockfish infected with *S. iniae* after thermal stress. The values represent the mean ± S.D of three replicates. Vertical bar denotes a standard error (n = 2).

Abrupt temperature fluctuations cause severe physiological stress in fish, which affects their immune system (Mariana *et al.*, 2019). Previous studies have shown that fish exposed to high temperature have protein damage, hormonal changes, and high mortality due to thermal stress (Basu *et al.*, 2002; Nakano *et al.*, 2014; Ahn *et al.*, 2019). Also, thermal stress mediate oxidative damages in liver, which affects survival rate of fishes (Roychowdhury *et al.*, 2020). However, this study shows that not only high-water temperature but also rapid fluctuations in water temperature have a significant impact on the survival rate of fish after the invasion by bacteria. Lillehaug *et al.* (1993) reported that rapid temperature changes cause severe physiological stress on fish, so any temperature change affects the immune system badly. In tilapia under temperature stress of 19°C and 35°C as of 27°C, tilapia decreased its resistance against *S. iniae* (Qiang *et al.*, 2013). Kayansamruaj *et al.* (2014) also reported that elevated temperature increases the severity of *Streptococcus agalactiae* in Nile Tilapia, *Oreochromis niloticus*. Their results suggest that the increase of *S. agalactiae* pathogenicity to fish induced by elevated temperature is associated with inflammatory responses such as IL-1β, TNF-α and COX-2, which may lead to acute mortality.

3. Immunological response analysis

In this study, the effect of thermal stress on the gene expression of heat shock protein 70 (HSP 70), Interleukin-1 beta (IL1β), Lysozyme g-type (Lyg) and Thioredoxin-like 1 (Txnl1) in the spleen and head kidney of black rockfish is shown in Figs. 3~6.

1) Heat shock protein 70 (HSP70)

The gene expression of HSP 70 significantly increased in spleen and head kidney of black rockfish exposed to thermal stress compared to the control ($P < 0.05$). The higher level of HSP 70 transcript was detected in the group of extreme water temperature changes (from 23°C to 28°C) than in the group exposed to high temperature (28°C) consistently. Also, our results showed that thermal stress was able to stimulate the level of HSP 70 gene expression within 12 hours in black rockfish (Fig. 3). HSP 70 played key roles in protein folding, protein targeting for degradation, apoptosis, etc. (Srinivasan *et al.*, 2018). It had been widely proved that HSP 70 was significantly induced by heat stress in aquatic species (Yan *et al.*, 2017; Huo *et al.*, 2019; Yu *et al.*, 2020). Similar result was displayed in this study with significantly upregulated HSP 70 expression after thermal stress. The enhanced expression of HSP 70 induced by stress was mainly involved in environmental stress protection, improving cell survival and disease resistance (Deane and Woo, 2005; Ryckaer *et al.*, 2009; Julia *et al.*, 2017).

2) Interleukin-1 beta (IL1β)

Likewise, the gene expression of IL1β significantly increased in head kidney of fish exposed to fluctuation water temperature within 12 hours ($P < 0.05$) (Fig. 4). However, after 24 hours of thermal stress, the immune responses, such as overexpression of HSP 70 and IL1β, were no longer observed. The Lyg also showed strong expression in head kidney after thermal stress (Fig. 5). In particular, high levels of Lyg were continuously observed in the head kidney of severely changed group (from 23°C to 28°C) even after 48 hours of thermal stress ($P < 0.05$), but no significant levels were observed in spleen even after 48 hours. These suppressions of immune response may lead to increase in the mortality rate of black rockfish infected with the *S. iniae* (Fig 2). The present results indicated a correlation between the immune response and survival rate against bacterial infection upon exposure to thermal stress.

The IL1 family is an expanding family of pro-inflammatory cytokines and an early cytokine in the inflammatory response with a cascade of effects, many of which is mediated through up- or down-regulation of other cytokines (Dunn *et al.*, 2001). Constitutive mRNA expression of IL1β in fish is

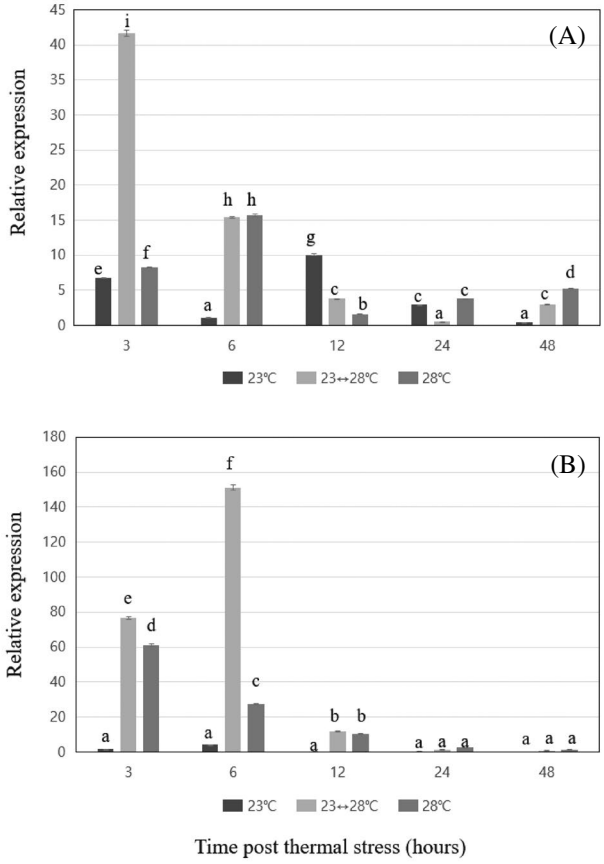

Fig. 3. Expression of the immune response gene; the heat shock protein 70 were measured in spleen (A) and head kidney (B) in the exposed fish to thermal stress for 48 hours by real-time PCR analysis. Ribosomal protein L17 gene was used as an internal control for real-time PCR and the relative expression level of 0 hours post-thermal stress was set to one. Deviation bars represented the standard errors of three experiments at each temperature point. The comparison among different time shocked by thermal stress was performed one-way ANOVA, followed by Duncan's multiple. Different superscripts indicate significant difference ($P<0.05$).

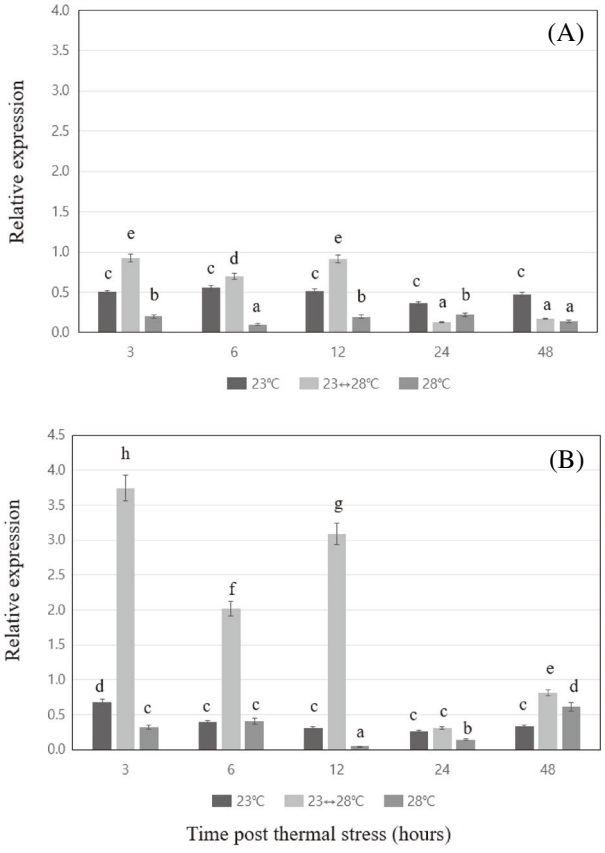

Fig. 4. Expression of the immune response gene; interleukin-1β were measured in spleen (A) and head kidney (B) in the exposed fish to thermal stress for 48 hours by real-time PCR analysis. Ribosomal protein L17 gene was used as an internal control for real-time PCR and the relative expression level of 0 hours post-thermal stress was set to one. Deviation bars represented the standard errors of three experiments at each temperature point. The comparison among different time shocked by thermal stress was performed one-way ANOVA, followed by Duncan's multiple. Different superscripts indicate significant difference ($P<0.05$).

primarily observed in the immune organs of head kidney and spleen (Engelsma *et al.*, 2001). In this study, although the expression of IL1β is observed both head kidney and spleen, a dramatic increase occurred only in head kidney after heat stress (Fig. 4). In trout, the Lipopolysaccharide-induced IL1 expression was shown to be temperature-dependent; a lower temperature in vitro resulted in lower expression (Engelsma *et al.*, 2002.). Also, injection of carp recombinant IL 1 could enhance the agglutinating antibody titers against bacteria, *Aeromonas hydrophila*, and the IL1β expression peaks approximately 2 h to 4 h after the start of stimulation (Zou *et al.*, 2000; Engelsma *et al.*, 2001).

3) Lysozyme g-type (Lyg)

As a non-specific immune factor of fish, the g-type lysozyme is closely related to the immune function of fish against bacterial infection. It is a natural endogenous antitoxin and helps improve the immunity of the body through its anti-bacterial, anti-viral, and anti-inflammatory activities (Shakoori *et al.*, 2019). Zhang *et al.* (2018a) reported that the Lyg expression in spleen, gill and intestine tissues of the largemouth bass, *Micropterus salmoides*, increased significantly under heat stress and *A. hydrophila* injection with the maximum levels attained at 12 and 24 hours. Furthermore, they all decreased significantly and the expression in gill returned to

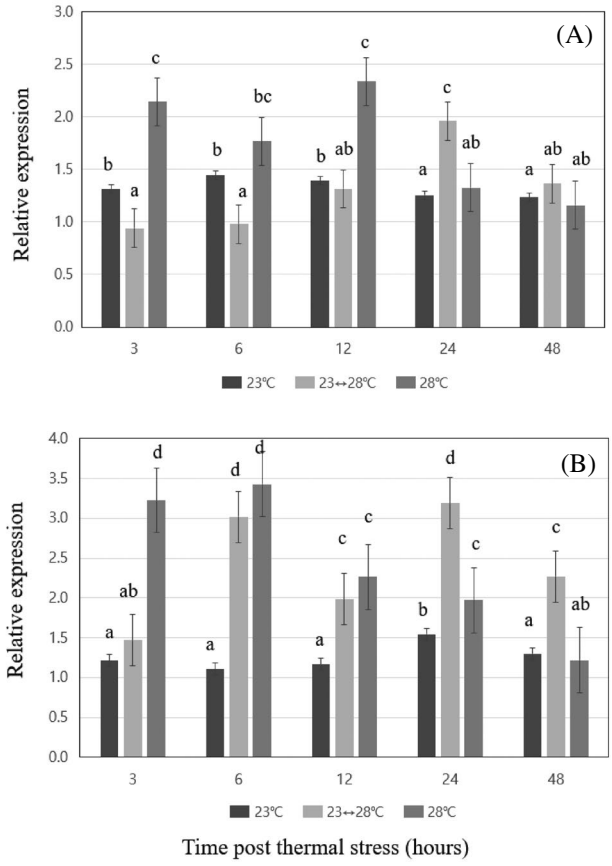

Fig. 5. Expression of the immune response gene; lysozyme g-type were measured in spleen (A) and head kidney (B) in the exposed fish to thermal stress for 48 hours by real-time PCR analysis. Ribosomal protein L17 gene was used as an internal control for real-time PCR and the relative expression level of 0 hours post-thermal stress was set to one. Deviation bars represented the standard errors of three experiments at each temperature point. The comparison among different time shocked by thermal stress was performed one-way ANOVA, followed by Duncan's multiple. Different superscripts indicate significant difference ($P<0.05$).

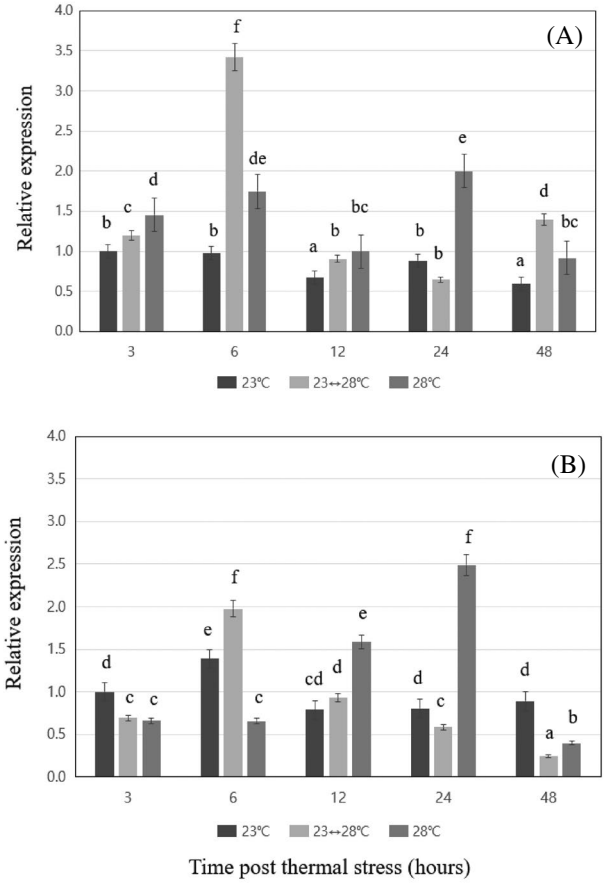

Fig. 6. Expression of the immune response gene; thioredoxin-like 1 were measured in spleen (A) and head kidney (B) in the exposed fish to thermal stress for 48 hours by real-time PCR analysis. Ribosomal protein L17 gene was used as an internal control for real-time PCR and the relative expression level of 0 hours post-thermal stress was set to one. Deviation bars represented the standard errors of three experiments at each temperature point. The comparison among different time shocked by thermal stress was performed one-way ANOVA, followed by Duncan's multiple. Different superscripts indicate significant difference ($P<0.05$).

nearly the basal value within 72 hours. They suggested that the Lyg gene perhaps played an important role in the immune responses against bacterial invasion. Their study, like our results, supports the suggestion that immune response actually decreases when the limit of stress that fish could endure is exceeded.

4) Thioredoxin-like 1 (Txnl1)

The immune related responses of the Txnl1 indicate the impact of pathological stimulation on thioredoxin pathway. ROS may interact with the biomolecules, leading to severe cell damage or cell death. The Txnl1 activity protects the cells from the damage via redox balance and it expressed in various tissues. In addition, the Txnl1 helps the organs to maintain cellular redox homeostasis related to antioxidant activity (Liyanage *et al.*, 2018). Furthermore, the thioredoxin transcripts of the scallop were up-regulated under bacterial stress *Vibrio parahaemolyticus* in scallop (Zhang *et al.*, 2018b), and may produce higher Txnl1 to eliminate the generated ROS from harmful invaders (Liyanage *et al.*, 2018). Indeed, it is well known that heat stress causes oxidative stress, resulting in increased disruption of the balance of antioxidant enzymes (Mujahid *et al.*, 2005, 2007; Montilla *et al.*, 2014). In this study, after thermal stress, although the overexpression of Txnl1 were observed in several experimental groups, no

regularities or a series of trends in its expression were found in both the kidney and spleen (Fig. 6). In the study of Duan *et al.* (2018), the expression of the thioredoxin gene showed significant variations after 24-72 hours of thermal stress in whiteleg shrimp, *Litopenaeus vannamei*. They insisted that thermal stress disrupt the balance between oxidants and antioxidants, causing a loss in compensatory mechanisms due to excessive ROS in shrimp.

CONCLUSION

In conclusion, thermal stress modulated the mRNA level of immune-related genes, such as HSP 70, IL1β, Lyg and Txnl1, and they may play an important role in the immune defense against bacteria challenge after thermal stress in the black rockfish. This study indicates that a high and sudden water temperature change affects immune responses and reduces the disease resistance in Korean rockfish. This provides basic information on whether the gene expression of immunological parameters changes the survival rate of black rockfish infected with bacterial disease in response to thermal stress.

ACKNOWLEDGEMENT

This work was financially supported by National Institute of Fisheries Science (R2023051).

REFERENCES

Agnew, W. and A.C. Barnes. 2007. *Streptococcus iniae*: an aquatic pathogen of global veterinary significance and a challenging candidate for reliable vaccination. *Veterinary Microbiology* **122**(1-2): 1-5.

Ahn, C.H., S. Lee, H.M. Song, H.R. Park and J.C. Joo. 2019. Assessment of water quality and thermal stress for an artificial fish shelter in an urban small pond during early summer. *Water* **11**(1): 139.

Alborali, L. 2006. Climatic variations related to fish diseases and production. *Veterinary Reseach Communications* **30**: 93-37.

Basu, N., A.E. Todgham, P.A. Ackerman, M.R. Bibeau, K. Nakano, P.M. Schulte and G.K. Iwama. 2002. Heat shock protein genes and their functional significance in fish. *Gene* **295**: 173-183.

Bowden, T.J. 2008. Modulation of the immune system of fish by their environment. *Fish and Shellfish Immunology* **25**: 373-383.

Boyd, M.A., S.M. Tennant, V.A. Saague, R. Simon, K. Muhsen, G. Ramachandran, A.S. Cross, J.E. Galen, M.F. Pasetti, M.M. Levinea. 2014. Serum Bactericidal Assays to Evaluate Typhoidal and Nontyphoidal Salmonella Vaccines. *Clinical and Vaccine Immunology* **21**(5): 712-721.

Choi, H.S., B.Y. Jee, M.Y. Cho, M.A. Park. 2010. Monitoring of pathogens on the cultured Korean rockfish *Sebastes schlegeli* in the marine cages farms of south sea area from 2006 to 2008. *Journal of Fish Pathology* **23**(1): 27-35.

Cooper, E.A. and R.B. Haines. 1928. The Influence of Temperature on Bactericidal Action. *Jornal of Hygene Cambination* **28**: 163.

Deane, E.E., N.Y. Woo. 2005. Cloning and characterization of the hsp70 multigene family from silver sea bream: modulated gene expression between warm and cold temperature acclimation. *Biochemistry and Biophysical Research Communications* **330**: 776-783.

Dittmar, J., H. Janssen, A. Kuske, K. Joachim. 2013. Heat and immunity: an experimental heat water alters immune functions in three-spined sticklebacks (*Gasterosteus aculeatus*). *Jouranl of Animal Ecology* **83**(4): 744-757.

Duan, Y., Q.S. Liu, Y. Wang, J.S. Zhang and D. Xiong. 2018. Impairment of the intestine barrier function in *Litopenaeus vannamei* exposed to ammonia and nitrite stress. *Fish and Shellfish Immunology* **78**: 279-288.

Dunn, E., J.E. Sims, M.J. Nicklin and L.A. O'Neill. 2001. Annotating genes with potential roles in the immune system: six new members of the IL-1 family. *Trends of Immunology* **22**: 533-536.

Engelsma, M.Y., M.O. Huising, W.B. van Muiswinkel, G. Flik, J. Kwang, H.F.J. Savelkoul, B.M.L.Verburg-van Kemenade. 2002. Neuroendocrine-immune interactions in fish: a role for interleukin-1. *Veterinary Immunolgoy and Pathology* **87**(3-4): 467-479.

Engelsma, M.Y., R.J. Stet, H. Schipper, B.M. Verburg-van Kemenade. 2001. Regulation of interleukin 1 beta RNA expression in the common carp, *Cyprinus carpio* L. *Development of Comparative Immunology* **25**(3): 195-203.

Harvell, D., S. Altizer, I.M. Cattadori, L. Harrington, E. Weli. 2008. Climate change and wildlife diseases: when does the host matter the most. *Ecology* **27**: 467-484.

Huo, D., L. Sun, L. Zhang, H. Yang, S. Liu, J. Sun and F. Su. 2019. Time course analysis of immunity-related gene expression in the sea cucumber *Apostichopus japonicus* during exposure to thermal and hypoxic stress. *Fish and Shellfish Immunology* **95**: 383-390.

Jeun, M.J., Y.J. Choe, Y.H. Kim, D. Ray, S.Y. Yoon and H.Y. Kang. 2017. Effect of different temperature on *Streptococcus iniae* FP5228. 2017. Int. Meet. Microbiol. Soc. Korea, p.230.

Julia, I., T. Barnoud, G. Zhang, T. Tian, Z. Wei, M. Herlyn, M.E. Murphy and D.L.George. 2017. Inhibition of stress-inducible HSP70 impairs mitochondrial proteostasis and function. *Oncotarget* **8**(28): 45656.

Jung, S.H., H.S. Choi, W.D. Jeung, M.S. Kim, M.G. Kwon, J.S. Seo, J.Y. Hwang, S.R. Kim, Y.R. Cho, J.D. Kim and M.A. Park. 2012. Monitoring of bacteria and parasites in cultured olive flounder, block rockfish, red sea bream and shrimp during summer period in Korea frmm 2007 to 2011. *Jouranl of Fish Pathology* **25**(3): 231-241.

Karvonen, A., P. Rintamaki, J. Jokela and E.T. Valtonen. 2010. Increasing water temperature and diseases risks in aquatic systems: climate change increases the risk of some, but not all, diseases. *International Journal fo Prasitology* **40**(13): 1483-14884.

Kayansamruaj, P., N. Pirarat, I. Hirono and C. Rodkhum. 2014. Increasing of temperature induces pathogenicity of Streptococcus agalactiae and the up-regulation of inflammatory related genes in infected Nile tilapia (*Oreochromis niloticus*). *Veterinary Microbiology* **172**(1-2): 265-271.

Kugapreethan, R., N. Umasuthan, Q. Wan, W.S. Thulasitha, C. Kim and J.H. Lee. 2017. Comparative analysis of two thioredoxin-like genes in black rockfish Sebastes schlegelii and their possible involvement in redox homeostasis and innate immune responses. *Development & Comparative Immunolgy* **67**: 43-56.

Lee, D.C., C.P. Park, Y.J. Yong, C.Y. Jeon, J.Y. Yang, Y.B. Hur, J.W. Kim and Cho K.C. 2013. A report on the 2012 mass summer mortalities of black rockfish, *Sebastes schlegeli* in the Southeast Sea, Korea. *Journal of Fish Pathology* **26**(3): 173-183.

Lee, D.C., M.W. Won, M.Y. Park, C.H. Sung and Jung S.H. 2018. An analysis of mass mortalities in aquaculture fish farms on the Southern coast in Korea. *Ocean Policy and Research* **33**: 1-16.

Lejeusne, C., P. Chevaldonn, C. Pergent-Martini, C.F. Boudouresque and T. Pérez. 2010. Climate change effects on a miniature ocean: the highly diverse, highly impacted Mediterranean Sea. *Trends of Ecology and Evolution* **25**: 250-260.

Lillehaug, A., A. Ramstad, K. Baekken and L. Reitan. 1993. Protective immunity in Atlantic salmon (*Salmo salar* L.) vaccinated at different water temperature. *Fish and Shell -fish Immunology* (3): 143-156.

Liman, M., W. Wang, L. Conghui, H. Yn, Z. Wang, X. Wang, J. Qi and Q. Zhang. 2013. Selection of reference genes for reverse transcription quantitative realtime PCR normalization in black rockfish (*Sebastes schlegeli*). *Marine Genomics* **11**: 67-73.

Livak, K.J. and T.D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**(4): 402-408.

Liyanage, D.S., W.K.M. Omeka, G.I. Codahewa and J.H. Lee. 2018. Molecular characterization of thioredoxin-like protein 1 (TXNL1) form big-belly seahorse *Hippocampus abdominalis* in response to immune stimulation. *Fish and Shellfish Immunology* **75**: 181-189.

Lonergan and J.T. Selsby. 2014. Heat stress causes oxidative stress but not inflammatory signaling in porcine skeletal muscle. *Temperature* **1**(1): 42-50.

Marcos-Lopez, M., P. Gale, B.C. Oidtmann and E.J. Peeler. 2010. Assessing the impact of climate change on disease emergence in freshwater fish in the United Kingdom. *Trans-bound Emerging Disease* **57**(5): 293-304.

Mariana, S., Alfons, B. Gamal. 2019. Impact of heat stress on the immune response of fishes. *Jornal of Survry in Fisheries Sciences* **5**(2): 149-159.

Montilla, S.I.R., T.P. Johnson, S.C. Pearce, D.G. Salmon, N.K. Gabler, J.W. Ross, R.P. Rhoads, L.H. Baumgard, S.M. Lonergan and J.T. Selsby. 2014. Heat stress causes oxidative stress but not inflammatory signaling in porcine skeletal muscle. *Temperature* **1**(1): 42-50.

Mu, C., S. Zhang, G. Yu, N. Chen., X. Li, H. Liu. 2013. Overexpression of small heat shock protein LimHSP16.45 in Arabidopsis enhances tolerance to abiotic stresses. *PloS One* **8**(12): e82264.

Mujahid, A., Y. Yoshiki, Y. Akiba, M. Toyomizu, 2005. Superoxide radical production in chicken skeletal muscle induced by acute heat stress. *Poultry Science* **84**: 307-314.

Mujahid, A., N.R. Pumford, W. Bottje, K. Nakagawa, T. Miyazawa, Y. Akiba and M. Toyomizu. 2007. Mitochondrial oxidative damage in chicken skeletal muscle induced by acute heat stress. *The Journal of Poultry Science* **44**: 439-445.

Nakano, T., M. Kameda, Y. Shoji, S. Hayashi, T. Yamaguchi and M. Sato. 2014. Effect of severe environmental thermal stress on redox state in salmon. *Redox Biology* **2**: 772-776.

Nho, S.W., G.W. Shin, S.B. Park, H.B. Jang, I.S. Cha, M.A. Ha, Y.R. Kim, Y.K. Park, R.S. Dalvi, B.J. Kang, S.J. Joh and T.S. Jung. 2009. Phenotypic characteristics of *Strptococcus iniae* and *Streptococcus parauberis* isolated form olive flounder (*Paralichthys olivaceus*). *FEMS Microbiology Letters* **293**(1): 20-27.

Qiang, J., H. Yang, H. Wang, M.D. Kpundeh and P. Xu. 2013. Interacting effects of water temperature and dietary protein level on hematological parameters in Nile tilapia juveniles, *Oreochromis niloticus* (L.) and mortality under *Streptococcus iniae* infection. *Fish and Shellfish Immunology* **34**: 8-16.

Rao, Y.V., B.K. Das, R. Jyotyrmayee and R. Chakrabarti. 2005. Effect of Achyranthes aspera on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. *Fish Shellfish Immunology* **20**(3): 263-273.

Roychowdhury, P., M. Aftabuddin and M.K. Pati. 2020. Thermal stress-induced oxidative damages in the liver and asso-

ciated death in fish, *Labeo rohita*. *Fish Physiol Biochem.* **47**(1): 21-32.

Ryckaer, J., F. Pasmans, E. Tobback, L. Duchateau, E. Decostere, F. Haesebrouck, P. Sorgeloos and P. Bossier. 2009. Heat shock proteins protect platyfish (*Xiphophorus maculatus*) from *Yersinia ruckeri* induced mortality. *Fish and Shellfish Immunology* **28**: 228-231.

Shakoori, M., S.H. Hoseinifar, H. Paknejad, V. Jafari, R. Safari, H.V. Doan and M.T. Mozanzadeh. 2019. Enrichment of rainbow trout (*Oncorhynchus mykiss*) fingerlings diet with microbial lysozyme: Effects on growth performance, serum and skin mucus immune parameters. *Fish and Shell -fish Immunology* **86**: 480-485.

Srinivasan, S.R., L.C. Cesa, X. Li, O. Julien, M. Zhuang, H. Shao, J.H. Chung, I. Maillrd, J.A. Wells, C.S. Duckett, J.E. Gestwicki. 2018. Heat shock protein 70 (Hsp70) suppresses RIP1-dependent apoptotic and necroptotic cascades. *Molecular Cancer Research* **16**(1): 58-68.

Zlotkin, A., S. Chilmonczyk, M. Eyngor, A. Hurvitz, C. Ghittino and A. Eldar. 2003. Trojan Horse Effect: Phagocyte-Mediated *Streptococcus iniae* Infection of Fish. *Infection Immunity* **71**(5): 2318-2325.

Zou, J., S. Bird, R. Minter, J. Horton, C. Cunningham and C.J. Secombes. 2000. Molecular cloning of the gene for interleukin-1b from *Xenopus laevis* and analysis of expression in vivo and in vitro. *Immunogenetics* **51**: 332-338.

Yan, J., X. Liang, Y. Zhang, Y. Li, X. Cao and J. Gao. 2017. Cloning of three heat shock protein genes (hsp70, hsp90α and hsp90β) and their expressions in response to thermal stress in loach (*Misgurnus anguillicaudatus*) fed with different levels of vitamin c. *Fish and Shellfish Immunology* **66**: 103-111.

Yu, H.H., C. Zhang, W. Xing, T. Li, G. Xu, Z. Ma, N. Jiang and L. Luo. 2020. Comparative study on the non-specific immune response and *hsp70* gene expression among three strains of koi (*Cyprinus carpio*) under acute heat stress. 18(November), 100461.

Zhang, C., J. Zhang, M. Liu and M. Huang. 2018a. Molecular cloning, expression and antibacterial activity of goose-type lysozyme gene in *Microptenus salmoides*. *Fish and Shellfish Immunology* **82**: 9-16.

Zhang, H., D. Cheng, H.O. Liu and H. Zheng. 2018b. Differential responses of a thioredoxin-like protein gene to *Vibrio parahaemolyticus* challenge in the noble scallop *Chlamys nobilis* with different total carotenoids content. *Fish and Shellfish Immunology* **72**: 377-382.