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Oxidative stress responses of golden and brown noble scallops *Chlamys nobilis* to acute cold stress

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ABSTRACT

The noble scallop *Chlamys nobilis* is an important edible marine bivalve that is widely cultivated in the sea of southern China. Unfortunately, the mass mortality of noble scallops frequently occurs during the winter months. The present study investigated the effects of acute cold stress (8 °C) to the physiological responses of polymorphic noble scallops, by assessing the *HSP70* gene expression, total carotenoid content (TCC), total antioxidant capacity (TAC), malondialdehyde (MDA) content, catalase (CAT) activity and superoxide dismutase (SOD) enzymatic activity in different tissues of golden and brown scallops. The results of the present study revealed that MDA, TCC and CAT increased drastically in most tissues in the early stage of acute cold stress (0–3 h), but TCC, SOD and CAT generally showed a downward trend. Within 3–6 h of acute cold stress, MDA content decreased in most tissues and the SOD content increased significantly in most tissues, while TCC and CAT remained at peak. After 6 h of acute cold stress, MDA content continued to increase in most tissues, while TCC, CAT, SOD and TAC decreased or remained at a lower level. For *HSP70* expression, up-regulation of the *HSP70* gene was observed only in mantle of brown scallops and hemolymph of golden scallops at 3 h and 24 h, respectively. The findings of the present study can better understand the physiological response of noble scallops to acute cold stress.

1. Introduction

Marine bivalve molluscs are exposed to dynamic environments with high fluctuations in environmental parameters. Water temperature is a major abiotic factor affecting the growth, productivity and geographical distribution of bivalves, particularly in temperate and subtropical waters [1–4]. Aquatic animals are able to sense transient fluctuations and seasonal variations in temperature, and respond to these changes by actively adjusting their physiological and biochemical activities to adapt to the ambient temperature regime [5–11]. However, when the water temperature exceeds the species-specific thermal tolerance range, the animal accumulates excess reactive oxygen species (ROS), including superoxide (O_2^-), hydroxyl radical (OH^-), and hydrogen peroxide (H_2O_2) [12]. Toxic ROS accumulate in all aerobic biological systems, which destroy many cellular components, leading to deleterious consequences and mortality.

The innate immune system is the first line of defense against stress in vertebrates, but the only line of defense for invertebrates [13]. Due to the lack of a specific immune system, both enzymes (e.g., catalase (CAT) and superoxide dismutase (SOD)) and non-enzymatic antioxidants (e.g., carotenoids) play a crucial role in the immunity of invertebrates [14]. SOD and CAT are key endogenous antioxidant enzymes, which in turn are involved in the detoxification of superoxide radicals: SOD converts superoxide to hydrogen peroxide, which is then detoxified to water and oxygen by CAT [15,16]. Carotenoids, on the other hand, are biologically active pigments that have a positive effect on the body condition [17], which stimulate the innate and adaptive components of vertebrate immunity and increase the efficiency of immune responses [18,19]. Carotenoids are potent antioxidants [20], which help endogenous enzymes (e.g., catalase, superoxide dismutase) detoxify free radicals produced by immunological activities [21].

The noble scallop *Chlamys nobilis* is an important edible marine bivalve belonging to the Pectinidae family, widely distributed in the coastal waters of Japan, Korea, Indonesia, Vietnam and the Southern Sea of China including Guangdong, Guangxi and Hainan Provinces [22]. Since the 1980s, noble scallop aquaculture has developed into a

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large-scale industry in China because of their obvious advantages of fast growth, short cultivation period, high profit, good taste and nutritious [23,24]. Unlike fish and shrimp aquaculture, bivalve farming is a self-regulating aquaculture that does not require additional feed (environmental friendly) [3,25]. Therefore, the commercial cultivation of noble scallops have been extensively carried out (6000-arces farming areas) along the Southern coastal areas of China including the Sanya area of Hainan province, Zhanjiang, Shenzhen, Shanwei, and Nan'ao island in Guangdong province, as well as in Dongshan, Zhangpu, Longhai, and Putian in Fujian province [26], with an annual production of about 0.1 million tons. However, when the sea water temperature in winter drops to below 15 °C (lowest recorded winter water temperature was 8 °C), a massive mortality of noble scallops often occur [27]. It is suspected that the mass mortality of noble scallops can be attributed to the accumulation of harmful reactive oxygen species (ROS) caused by lower temperature stress. In general, noble scallops not only display polymorphism in shell color (golden and brown), but also the polymorphism in tissues color (orange and white) [23]. Golden scallops have been reported to contain much higher total carotenoid content [28], and the winter survival rate (86–92%) is higher than that of brown scallops (63–69%) [29]. Therefore, understanding the physiological responses of polymorphic noble scallops to cold stress is necessary to better understand the biological and ecological effects of low temperature stress.

Heat shock proteins (HSP) are a family of proteins that play important roles in normal growth and development, helping organisms regulate stress responses [30–32]. When environmental factors deviate from the optimal biological range, the aerobic scope decline and trigger a series of stress responses, including the production of heat-shock proteins (HSP, a molecular chaperone that assists in refolding of stress-damaged proteins). When an organism is challenged by environmental stressors such as temperature, salinity, hypoxia, heavy metals, bacteria and etc., the mRNA expression level of HSP will increase, and the organism will resist adverse stress factors, thereby maintaining the homeostasis and survival of the cells [23,33,34]. HSP members are usually grouped according to their molecular weight and are divided into three main families: HSP90s, HSP70s and small HSPs [35]. Among these HSPs, the HSP70s are one of the most conserved and important protein families and have been studied in many marine bivalve species, including oysters [36–39], mussels [40,41], scallops [42,43] and clams [44,45].

To date, the physiological responses of oysters [36] and mussels [40] to cold stress have been documented. However, the detailed physiological responses of scallops to cold stress are still poorly understood. Since it is not unusual for scallops on farm to experience rapid and extreme temperature change of up to 10 °C in a few hours during the diurnal/tidal cycles [46,47], the present study aimed to study the response of golden and brown noble scallops (golden scallops containing significantly higher total carotenoid content (TCC) than brown scallops) to acute cold stress, by measuring TCC, total antioxidant capacity (TAC), malondialdehyde (MDA), CAT, SOD and *HSP70* gene expression. The findings of this study provide a better understanding of the physiological response of polymorphic noble scallops to acute cold stress.

2. Materials and methods

2.1. Experimental animals

The adult golden (60.63 ± 3.45 mm; 29.90 ± 5.10 g) and brown (60.78 ± 3.70 mm; 30.12 ± 5.49 g) noble scallops *Chlamys nobilis* (Fig. 1) used in this study were obtained from the Nan' Ao Marine Biology Experimental Station of Shantou University (Shantou, China). Before the experiment, scallops were maintained in aerated seawater at 20 °C for seven days. The water was completely changed daily, and diatom (*Nitzschia closterium f. minutissima*) and tetraselmis (*Platymonas subcordiformis*) were fed to scallops during the acclimation period and during the experiment.

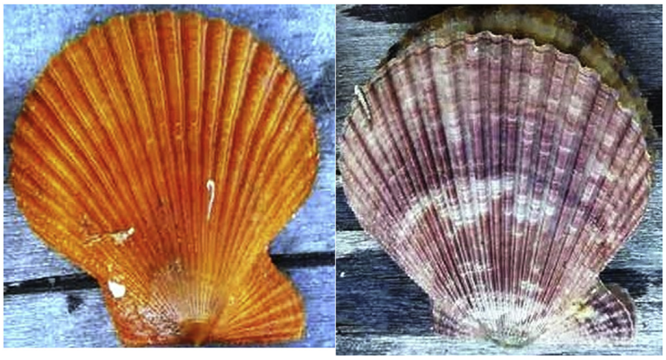

Fig. 1. Golden (Left) and brown scallops (Right).

2.2. Experimental design and samplings

Thirty golden and 30 brown scallops were transferred from the acclimation tank (20 °C) to the experimental tank (50 L) and maintained at 8.0 ± 0.5 °C for 36 h. Samples were taken at 0, 3, 6, 12, 24 and 36 h. Hemolymph was drawn from each scallop on ice using a disposable syringe (1 ml). The adductor, gills and mantle were also sampled and all samples were stored at −80 °C for further analysis. The animals were treated according to the "Regulations for the Administration of Affairs Concerning Experimental Animals" established by the Guangdong Provincial Department of Science and Technology on the Use and Care of Animals. Mortality and physiological changes in scallops, including shrinking and detachment of mantle were recorded in the experiments. Experiments were conducted in triplicates.

2.3. Determination of total carotenoid content (TCC)

The TCC in adductor, gills and mantle was determined according to the method of Zheng et al. [28]. All samples were freeze-dried in a vacuum freeze dryer (ScanVac CoolSafe, LaboGene, Denmark) and ground to homogenized powder in a mortar. Then, 0.01–0.03 g of the tissue powder was extracted with 1 ml of acetone with shaking (200 rpm) in the dark at 25 °C for 1 h. The extract was centrifuged at 5000 rpm for 5 min, and the supernatant was scanned from 400 to 700 nm in a spectrophotometer (UV2501PC, SHIMADZU, Japan). Finally, TCC (mg/g dry weight) was calculated at an absorbance of 480 nm using an extinction coefficient E (1%, 1 cm) of 1.900 [48].

2.4. Determination of malondialdehyde (MDA) content, total antioxidant capacity (TAC) and catalase (CAT) activity

The adductor, gills and mantle samples were homogenized separately in 0.86% ice-cold NaCl using a homogenizer (T18, IKA, Germany). The tissue homogenate was immediately centrifuged (12000 rpm, 10 min, at 4 °C) using a Sigma 3–18 K Centrifuge (Germany) and the supernatant was collected for MDA, TAC and CAT analysis using the corresponding detection kit (JianCheng, Nanjing, China) on a Microplate spectrophotometer (Epoch2, Bio Tek) at 532 nm, 414 nm and 280 nm, respectively, according to the manufacturer's instructions. Protein concentration (μg/mg) was measured at 562 nm by the Bradford method.

2.5. Determination of superoxide dismutase (SOD) enzymatic activity

The determination of SOD enzyme activity was carried out by using the NBT/RF method [49] with modifications. The sampled tissue was ground separately with a pestle in a cold mortar on ice. The grinding media consisted of 0.1 mol L^{-1} PBS. The homogenate was centrifuged at 3000 rpm for 20 min at 0 °C in a refrigerated centrifuge (SIGMA 3–18K, USA). The supernatant was retained as a crude SOD extract for further assay. The reaction mixture composed of 54 ml of 14.5 mmol L^{-1} $_{DL-}$ methionine solution, 2 ml of 0.3 mmol L^{-1} EDTA, 2 ml of 2.25 mmol L^{-1} NBT, and 2 ml of 60 μmol L^{-1} riboflavin solution. Subsequently, 3 ml of the reaction mixture solution was added to 1 ml of the crude extract. A mixture without extract was used as control. The mixture was illuminated in a glass test tube in a light incubator (40 μmmol photons $m^{-2}s^{-1}$) for 10 min, and then the reduced NBT was measured spectrophoto-metrically at 560 nm. The same solution that is not exposed to light is blank. The reaction was initiated and terminated by turning the light on and off. One unit of enzyme activity is defined as the amount of enzyme that has a 50% inhibition of NBT reduction. The values were calculated as units/mg protein.

2.6. The mRNA expression analysis of HSP70

Total RNA was extracted from gills, hemolymph and mantle using Trizol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The extracted RNA was then quantified by Nanodrop (2000) (Thermo Scientific) and checked for the integrity with Angilent 2100 Bioanalyzer (Agilent Technologies). The mRNA was purified with Dynabeads® mRNA DIRECTTM Micro Kit (Invitrogen) following the manufacturer's instructions. cDNA was synthesized using the Prime Script RT reagent Kit with gDNA Eraser (TaKaRa). The transcription level of *HSP70* was determined by qPCR on a Light Cycler®480 (Roche) using the SYBR® Premix Ex Taq™ II Kit (Perfect Real Time) (Takara, Japan) according to the manufacturer's protocol and the *β*-actin mRNA was used as internal control. The gene-specific primer sequence and the *β*-actin primer sequence are shown in Table 1. Amplification was performed under the following conditions: 95 °C for 1 min followed by 40 cycles at 95 °C for 10 s and 60 °C for 30 s, the melting curve was analysed from 65 °C to 95 °C, and the cooling step was 40 °C for 10 min. Melting curve analysis of amplification products was performed at the end of each PCR reaction to confirm that only one PCR product was amplified and detected, and the program was set as: 95 °C for 15 s, 60 °C for 30 s, 95 °C for 15 s. Each qPCR experiment was performed in triplicates. Relative mRNA expression value of *CnHSP70* was determined using the $2^{-\Delta\Delta CT}$ algorithm with *β*-actin from *Chlamys nobilis* as the internal control [50]; Livak and Schmittgen, 2001).

2.7. Statistical analysis

TCC, TAC, MDA, CAT and SOD data were expressed as mean ± S.D, while *HSP70* data was expressed as relative expression level (mean ± S.D). Significant differences ($P < 0.05$) of variables among tissues and between strains were analysed by one-way Analysis of Variance (ANOVA) and independent T-test, respectively. Prior to analyses, all variables were tested for normality and homogeneity of variances. All statistical analyses were performed on a SAS system for Windows (SAS 8.0, SAS Institute Inc., Cary, NC, USA) and the significance of all analyses was set to $P < 0.05$ unless noted otherwise. Correlation coefficients among TAC, TCC, CAT, MDA and SOD were estimated using Pearson regression.

Table 2
Physiological change in golden and brown scallops under acute cold stress.

Time (h)	Cumulative mantle shrink (%)		Cumulative mantle detached from shells (%)		Cumulative mortality rates (%)	
	G	B	G	B	G	B
3	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0
12	20.0	22.9	20.0	37.1	0.0	0.0
24	100.0	100.0	100.0	100.0	0.0	1.4
36	100.0	100.0	100.0	100.0	0.0	1.4

G = Golden scallops; B = Brown scallops.

3. Results

3.1. Scallops mortality and physiological changes in acute cold stress

Under acute cold stress (8 °C), golden and brown scallops began to exhibit physiological changes, including shrinking and detaching of mantle at 12 h. At this time, the percentage of lesion scallops was significantly higher ($P < 0.05$) in brown scallops (37.1%) than that of golden scallops (20.0%). In the longer period of acute cold stress exposure (24 and 36 h), shrinking and detaching of the mantle were observed in all scallops (100%). The mortality of golden scallops was not observed throughout the experiment. However, 1.4% mortality was observed in brown scallops at 24 h (Table 2).

3.2. Effects of acute cold stress on total carotenoid content (TCC) in tissues of golden and brown scallops

The effect of acute cold stress on TCC in adductor, gills and mantle of golden and brown scallops is illustrated in Fig. 2. In general, all tested tissues of golden and brown scallops responded rapidly to acute cold stress within the first 6 h, increasing TCC by 1.2–1.7 times ($P < 0.05$). The TCC was then declined but was still above the control level (0 h). The TCC level in tissues of golden scallops (88–274 μg/g) was significantly higher ($P < 0.05$) than that the respective tissues in brown scallops (22–76 μg/g).

3.3. Effects of acute cold stress on MDA, TAC, CAT and SOD in tissues of golden and brown scallops

Figs. 3–6 show the effects of acute cold stress on MDA, TAC and CAT in the adductor, gills and mantle of golden and brown scallops. The changes of MDA content in all examined tissues of golden and brown scallops showed a similar trend, in which MDA content increased drastically ($P < 0.05$) in the first 3 h, then decreased drastically at 6 h, and then increased again thereafter, with MDA content always higher than control (0 h), except for gills of golden scallops at 6 and 12 h. Moreover, the MDA content in golden scallops was significantly lower ($P < 0.05$) than that in brown scallops.

The TAC in golden scallops is generally higher ($P < 0.05$) than that the TAC in brown scallops. In all examined tissues of golden scallops, TAC decreased drastically ($P < 0.05$) in the first 3 h, and then showed a consistent declining trend. In brown scallops, TAC in the adductor showed a sharp increased ($P < 0.05$) in the first 6 h, followed by a

Table 1
Primers used in present study.

Primer	Sequence (5′-3′)	Amplification efficiency (%)	Melting temperature (°C)	Product Size (bp)	Function
HSP70RTF	ACGAGGGAGAACGAGCAATG	96	59.30	60	RT primer
HSP70RTR	TCTTGTCAACGGCAGAAACG	96	61.52	60	RT primer
β-actin F	CAAACAGCAGCCTCCTCGTCAT	98	60.58	62	RT primer
β-actin R	CTGGGCACCTGAACCTTTCGTT	98	60.40	62	RT primer

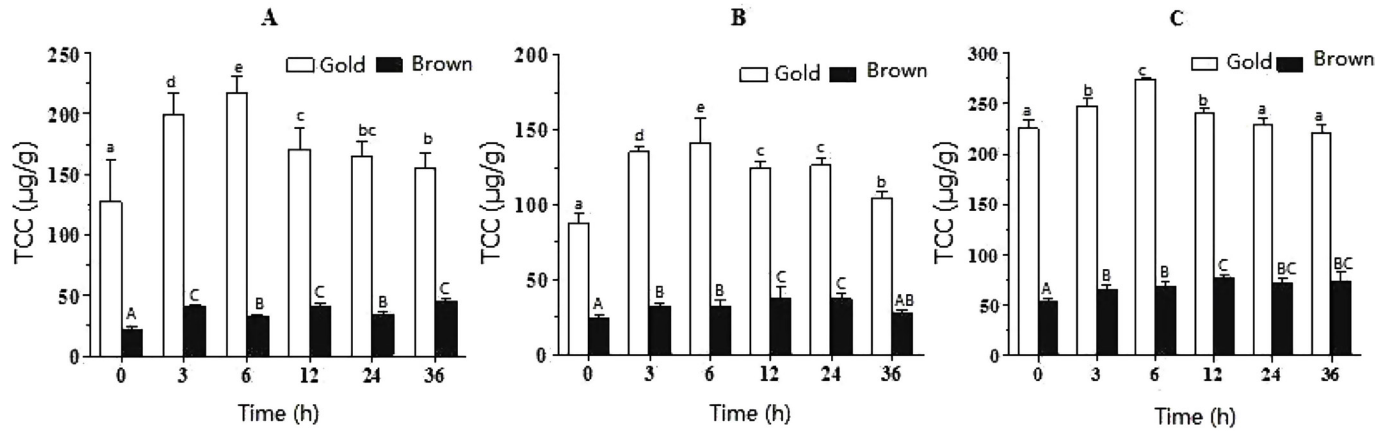

Fig. 2. Effects of acute cold stress on TCC in (A) adductor muscle, (B) gills and (C) mantle of golden and brown scallops. Values were expressed as mean ± SD (n = 3), different letters (uppercase = brown scallops; lowercase = golden scallops) indicate significant difference (P < 0.05).

sharp declined at 12 h, and then remained at a level above the control level. In the gills of brown scallops, we observed a delayed decline in TAC at 12 h, while the TAC in mantle of brown scallops was fluctuated at the level slightly higher than the control throughout the acute cold stress experiment.

The CAT activity of adductor and gills of golden and brown scallops was statistically insignificant ($P > 0.05$). However, the CAT activity of the golden scallop mantle was significantly higher ($P < 0.05$) than that of brown scallops. Typically, CAT activity increased drastically during the first 3–6 h and then declined below the control level. The CAT activity in all examined tissues peaked at 6 h, except for the adductor and gills of brown scallops, which responded faster and peaked at 3 h.

The SOD activity of adductor of golden scallops and all examined tissues of brown scallops were fluctuated at a level comparable to or slightly lower than the control. In the gill and mantle of golden scallops, the SOD activity at 6 h was significantly higher than ($P < 0.05$) that the control level.

3.4. Effects of acute cold stress on HSP70 expression level in tissues of golden and brown scallops

The effect of acute cold stress on the *HSP70* expression level in tissues of golden and brown scallops is illustrated in Fig. 7. Except for the mantle of brown scallops and hemolymph of both golden and brown scallops, the expression levels of *HSP70* in all tissues were significantly lower than control (0 h) under acute cold treatment. In the hemolymph, we observed delayed induction of *HSP70* expression levels at 24 h.

3.5. Correlation coefficients among TCC, TAC, CAT, MDA, SOD and HSP70 in golden and brown scallops

The results of Pearson's correlation test among TCC, TAC, CAT, MDA, SOD and *HSP70* in golden and brown scallops are summarized in Table 3 and Table 4, respectively. In golden scallops, the TCC was positively associated with the expression level of *HSP70* gene ($r = 0.49$, $P < 0.05$), but negatively correlated with MDA ($r = -0.36$, $P < 0.05$). Moreover, SOD responded positively to TAC ($r = 0.39$, $P < 0.05$), CAT ($r = 0.8$, $P < 0.05$) and MDA ($r = 0.39$, $P < 0.05$). In brown scallops, CAT was positively correlated with TAC ($r = 0.75$, $P < 0.05$) and MDA ($r = 0.37$, $P < 0.05$).

4. Discussions

In the aquatic environment, temperature is one of the main sources of stress that induces oxidative stress and affects the physiological responses of bivalve cells [40,51]. Previous studies have shown that cold stress induces the accumulation of reactive oxygen species (ROS), including superoxide, hydrogen peroxide and hydroxyl radicals, where elevated ROS concentrations might damage cellular structures and macromolecules, leading to cell death [40,52,53]. In the present study, at some time points of acute cold treatment, TCC, SOD (in gill of golden scallops) and CAT levels were all higher than the control, which means that TCC, SOD and CAT contribute to the acute cold tolerance of noble scallops [26]. Antioxidant systems are important regulatory mechanisms for the oxidization/reduction balance of organisms [54]. As an overall measure of the level of antioxidants in an organism, TAC

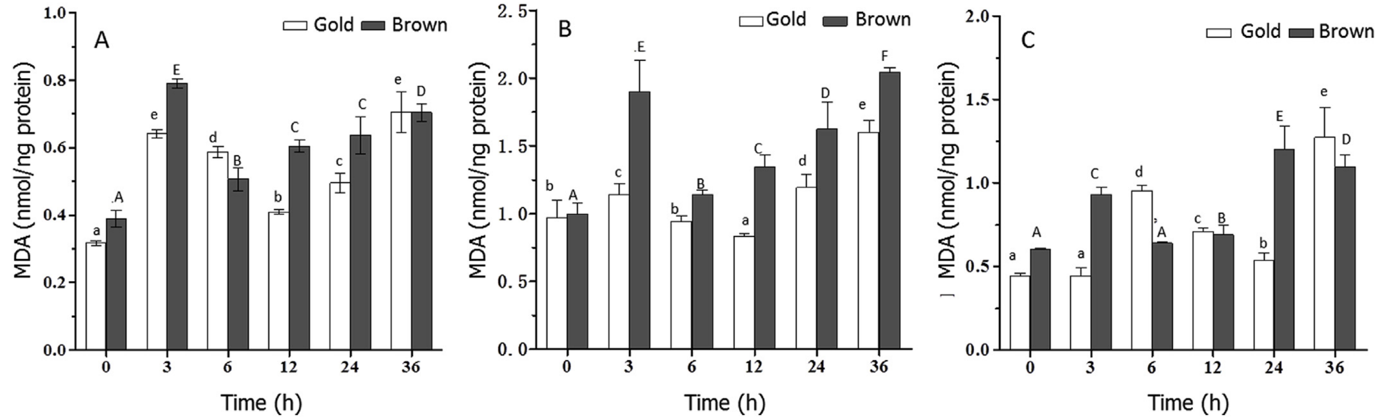

Fig. 3. Effects of acute cold stress on MDA content in (A) adductor muscle, (B) gills and (C) mantle of golden and brown scallops. Values were expressed as mean ± SD (n = 3), different letters (uppercase = brown scallops; lowercase = golden scallops) indicate significant difference (P < 0.05).

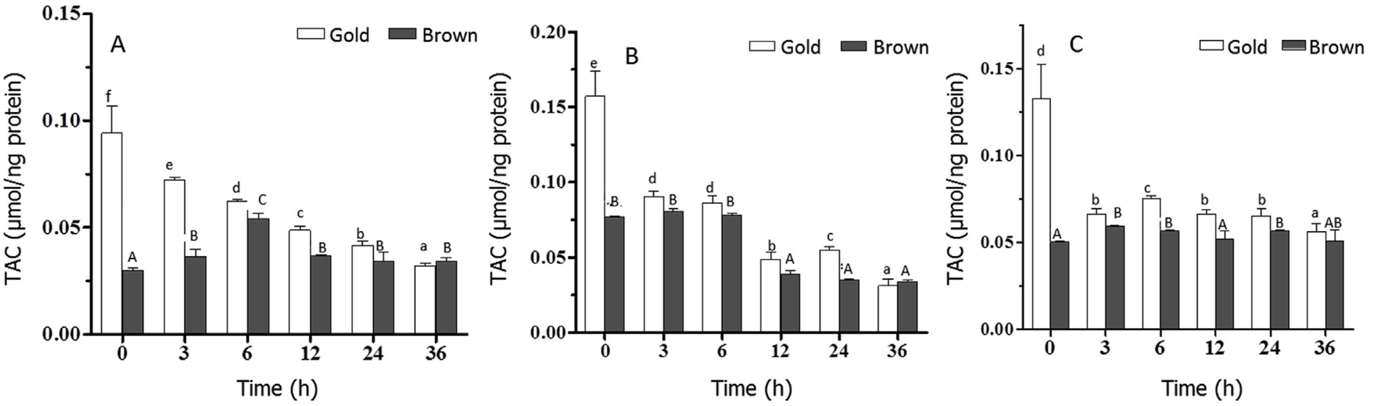

Fig. 4. Effects of acute cold stress on TAC content in (A) adductor muscle, (B) gills and (C) mantle of golden and brown scallops. Values were expressed as mean ± SD (n = 3), different letters (uppercase = brown scallops; lowercase = golden scallops) indicate significant difference ($P < 0.05$).

represents the antioxidant capacity of the organism. In the present study, we recorded a declined in TAC for noble scallops under acute cold stress, which contradicts the generally accepted theory; under stress conditions, TAC is initially increased to eliminate free radicals produced in organisms and returned to baseline after recovery, followed by decreased antioxidant enzymes activity over time [55]. However, the findings of the present study are consistent with the observations reported by Lan et al. [26], where the TAC of scallops in Hunter Bay and Baisha Bay decreased with increasing mortality and severe damage to noble scallops, suggesting that the lesion have weakened antioxidant capacity.

Malondialdehyde (MDA) is an important indicator of membrane system injuries and deterioration of cellular metabolism caused by environmental stress [56]. The results of the present study revealed that MDA level in scallops increased drastically at the beginning (0–3 h) of acute cold stress (8 °C), indicating that scallop tissues have high degree of stress or cell damage. At the same time, TCC and CAT activity increased dramatically in response to acute cold stress, accompanied by a sharp decline in SOD and TAC. Similarly, Wang et al. [40] demonstrated that MDA and CAT levels were elevated in *Mytilus galloprovincialis* gills after cold stress, but SOD levels were lower than controls. Under acute cold stress of 3–6 h, the MDA level in scallop tissues reduced drastically, which can be explained by the high TCC and CAT levels. At the same time, the SOD activity of gills and mantle also significantly increased, which could further promote the cold tolerance of noble scallops under acute cold stress. The TCC in noble scallops was then dropped significantly between 6 and 12 h, indicating that substantial consumption of carotenoids can counteract oxidative stress [26]. This observation can also be partly explained by the fact that the digestive system of noble scallops does not function well at low temperatures, thus reducing the absorption and accumulation efficiency of carotenoids in food. As a result, MDA level in noble scallops increased, and both golden and brown scallops began to exhibit physiological changes, including shrinking and detaching of mantle. Between 12 and 36 h, as TCC, CAT, SOD and TAC continue to drop, the MDA in noble scallops continues to increase, and then more and more scallops are adversely affected by acute cold stress (mantle detached in all scallops). This observation was consistent with the finding of Yang et al. [57], who reported stress injury gradually become irreparable in oyster when the stress treatment prolonged to 24 h.

The golden and brown scallops described in this paper have the same genetic background and are cultivated under the same environment (Nan'ao Island, Guangdong Province, China). The only difference between them is that golden scallops contain TCC significantly higher than brown scallops [29]. In the present study, higher MDA but lower TAC in brown scallops than golden scallops indicated higher levels of cell damage in brown scallops under acute cold stress (8 °C). This observation was supported by the negative correlation between TCC and MDA, which was only recorded in golden scallops, but not in brown scallops. Furthermore, the expression levels and patterns of *HSP70* are closely related to stress tolerance in organisms [58]. An interesting observation in the present study was that the relative expression levels of *HSP70* in all brown scallop tissues, except for at 6 h in hemolymph and at 3, 6 and 12 h in mantle, were lower than the golden scallops, suggesting that the golden scallops were more resistant to acute cold stress. This observation is consistent with the findings of Zheng et al.

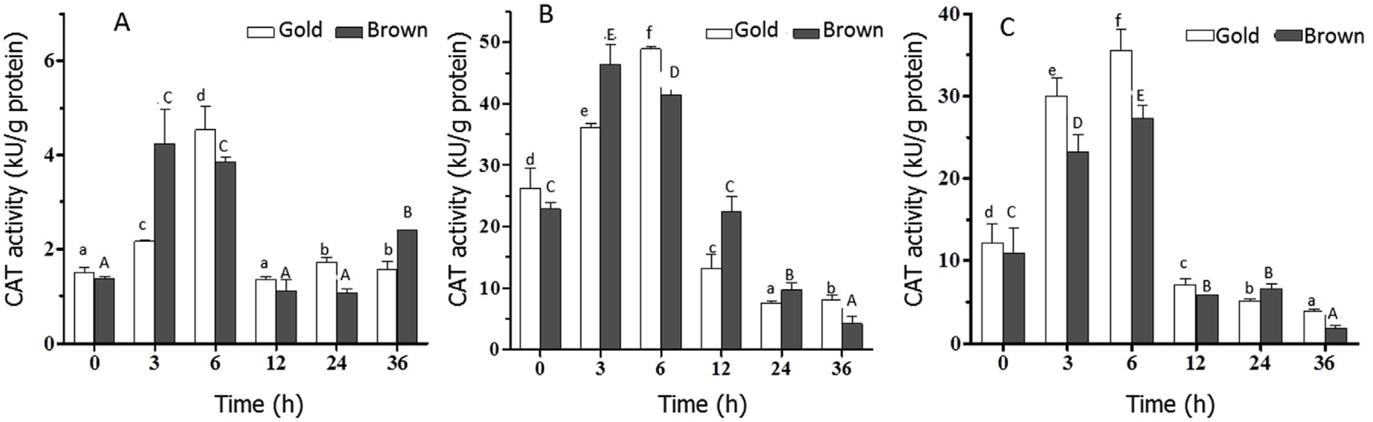

Fig. 5. Effects of acute cold stress on CAT activity in (A) adductor muscle, (B) gills and (C) mantle of golden and brown scallops. Values were expressed as mean ± SD (n = 3), different letters (uppercase = brown scallops; lowercase = golden scallops) indicate significant difference ($P < 0.05$).

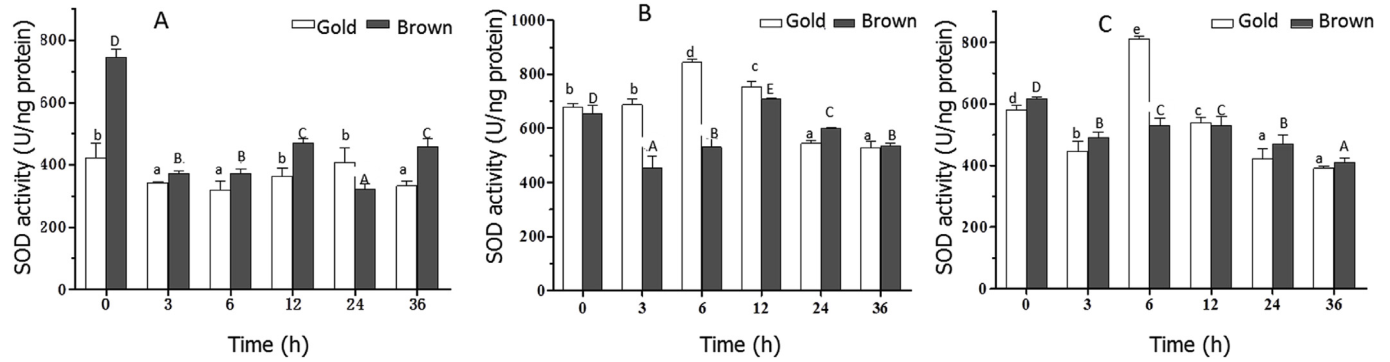

Fig. 6. Effects of acute cold stress on SOD activity in (A) adductor muscle, (B) gills and (C) mantle of golden and brown scallops. Values were expressed as mean ± SD (n = 3), different letters (uppercase = brown scallops; lowercase = golden scallops) indicate significant difference (P < 0.05).

[29], who reported that the mortality rate in golden scallops (8–14%) was lower than that of brown scallops (31–36%) during the winter months. In addition, the present study recorded a positive correlation between TCC and *HSP70* expression levels. Since carotenoids in noble scallops have been shown to up-regulate gene expression of several immune related genes, including the thioredoxin-like protein (*TRX*) gene [59] and toll-like Receptor (*TLRs*) gene [60]. Therefore, we do not rule out the possibility that carotenoids may affect the expression of *HSP70* in noble scallops.

The *HSP70* is involved in a variety of cellular processes, including protein folding/unfolding, translocation, targeting, degradation, and protein complex remodelling, where the expression of *HSP70* is triggered by a stimulus sufficiently strong to damage proteins [61]. In general, under acute cold stress, the transcription level of *HSP70* in gills of golden and brown scallops was depressed. Similar observations have been documented in *M. galloprovincialis* [40] and *Crassostrea gigas* [36], in which the expression level of *HSP7*0 mRNA in the gill tissue is reduced in the cold shock trials. It is known that cold shock treatment slows the activity of proteins, disrupt the integrity of essential organelles, and inhibits important processes such as transcription and mRNA translation [62]. Since gills have experienced the greatest exposure to water movement, it has the highest cold and oxidation stress (as indicated by MDA level in the present study; 1.0 to 2.0 in gills versus 0.3 to 1.2 in adductor and mantle), thus, we speculate that cold stress may have a negative impact on the transcription of *HSP70* in gills. This inference is consistent with the views of Wang et al. [40] who reported that *HSP70* synthesis in gills of *M. galloprovincialis* was inhibited or could not be induced in cold stress (9 °C). Interestingly, the results of the present study indicate a dramatic increase in the transcriptional level of *HSP70* in the hemolymph of golden and brown scallops at 24 h. Similar observations have been documented in the *Ruditapes philippinarum* [2] and *C. gigas* (David et al., 2005), in which *HSP70* gene expression is inducible in other tissues under cold stress.

Table 3
Pearson's correlation coefficients among TCC, TAC, CAT, MDA and SOD in golden scallops.

	TCC	TAC	CAT	MDA	SOD	*HSP70*
TCC		−0.08	−0.01	**−0.36**	−0.19	**0.49**
TAC			**0.41**	−0.14	**0.39**	0.21
CAT				0.26	**0.80**	0.18
MDA					**0.39**	0.13
SOD						0.09

Number in bold indicating significant different (P<0.05)

Table 4
Pearson's correlation coefficients among TAC, TCC, CAT, MDA and SOD in brown scallops.

	TCC	TAC	CAT	MDA	SOD	*HSP70*
TCC		0.12	−0.06	−0.19	−0.22	**0.33**
TAC			**0.75**	0.22	−0.01	0.17
CAT				**0.37**	0.19	0.12
MDA					0.10	0.05
SOD						0.03

Number in bold indicating significant different (P<0.05)

5. Conclusion

In a nutshell, this study shows that golden scallops present a higher cold stress tolerance than brown scallops, probably due to the higher TCC in golden scallops. The results also revealed a tissue-specific effect of cold stress in the expression of HSP70, where acute cold stress

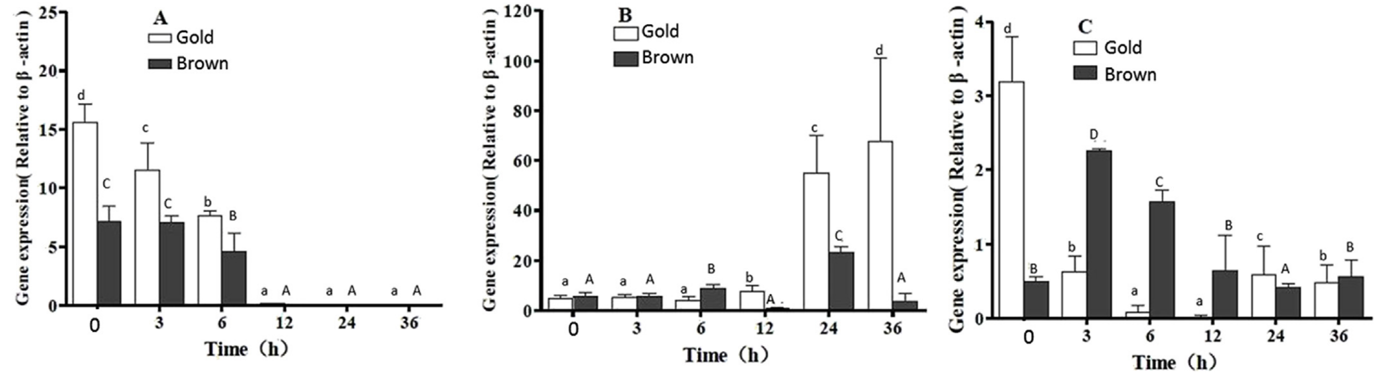

Fig. 7. Effects of acute cold stress on relative *HSP70* expression in (A) gills, (B) hemolymph and (C) mantle of golden and brown scallops. Values were expressed as mean ± SD (n = 3), different letters (uppercase = brown scallops; lowercase = golden scallops) indicate significant difference (P < 0.05).

inhibited expression in gills, but induced expression in hemolymph and mantle. Altogether, these results provide a better understanding of the strain-specific responses of golden and brown scallops to cold stress, and suggest that golden scallops are more suitable for aquaculture in winter and can withstand acute cold stress effects for at least 8 h.

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