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Exposure to domoic acid causes oxidative stress in bay scallops *Argopecten irradians*

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Abstract

The neurotoxin domoic acid (DA) is produced by *Pseudo-nitzschia* spp. during harmful algal blooms and is linked to amnesic shellfish poisoning. This toxin is particularly problematic in the culture of bivalves for human consumption. In this study, we exposed bay scallops to 20, 40, and 60 ng/ml DA and examined toxin accumulation and stress response at 3, 6, 12, 24, 36, and 48 h of exposure. Oxidative stress was determined by measuring the levels of superoxide dismutase (SOD), catalase (CAT), heat shock protein 90 (HSP90), and metallothionein (MT) in the digestive diverticula and/or hemolymph and by determining H_2O_2 concentration in hemolymph. We also observed histological changes to gills following exposure to DA in order to determine changes in melanization. We found that scallops accumulated a maximum of 2.61 ng/ml DA in their digestive diverticula when exposed to 60 ng/ml. Stress indices rose under higher DA concentrations, and HSP levels increased under exposure to higher concentrations of DA. However, mRNA expression and activity of other stress parameters showed peaks at different times during exposure, with subsequent declines. Epithelial melanization of gills indicated a strong positive response to DA at 60 ng/ml for 48 h ($P < 0.05$). These results indicate that exposure to DA induces oxidative stress, disrupts metabolism, and has negative effects on the defense systems of bay scallops.

Keywords Antioxidant · *Argopecten irradians* · Domoic acid · Heat shock protein · Metallothionein · Oxidative stress

Introduction

The range of harmful algae blooms (HABs) is increasing in marine ecosystems due to pollution and global warming (Parsons et al. 2002; Anderson 2014). In HABs, toxins are produced as secondary metabolites by phytoplankton at the bottom of the food web; these are then consumed by filter-feeding organisms where they can accumulate. The transfer of these toxins upwards through the food web has the potential to ultimately cause harm to humans who consume seafood (Perl et al. 1990; Hallegraeff 2010). Consequently, HABs can cause considerable economic losses to the aquaculture industry (Hoagland and Scatasta 2006).

The main marine phycotoxin substances causing poisoning in humans induced by algal toxicity in shellfish can be categorized as paralytic shellfish poison (PSP), neurotoxic shellfish poison (NSP), amnesic shellfish poison (ASP), diarrhetic shellfish poison (DSP), azaspiracid shellfish poison (AZP), and ciguatera fish poison (CFP) (Van Dolah 2000; James et al. 2010). All of these toxins accumulate in the tissues of shellfish, and people eating shellfish contaminated with any of these toxins can become ill (Hallegraeff 2010; James et al. 2010). Domoic acid (DA), a neurotoxin produced by some members of the diatom genus *Pseudo-nitzschia*, is associated with ASP in humans (Bates et al. 1989; Todd 1993). Blooms of *Pseudo-nitzschia* have been increasing in frequency in recent years, and DA is also widely distributed in seawater (Trainer et al. 2012; Lelong et al. 2012). Studies on the physiological responses of shellfish to toxic concentrations of DA are required to ensure

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that cultured shellfish yield is maintained to meet economic demand.

Scallops readily accumulate toxins in their tissues (Cranford 2006; Hannam et al. 2009). Reactive oxygen species (ROS) are generated due to the accumulation of toxins, such as phycotoxin (Chi et al. 2016), and the ROS generated are transported via hemolymph to cause oxidative damage throughout the body, adversely affecting survival (Chen et al. 2007; Gao et al. 2008). In scallops, superoxide dismutase (SOD) is the first and most important line of defense against ROS. This enzyme protects the tissue from oxidative damage by partitioning ROS into secondary components, including hydrogen peroxide (H_2O_2), which is further broken down into oxygen and water molecules by another antioxidant enzyme, catalase (CAT) (Kashiwagi et al. 1997). Consequently, SOD and CAT are widely used as biomarkers for measuring oxidative stress in scallops (Chi et al. 2014; Frantzen et al. 2016). Metallothionein (MT) is also involved in the response to oxidative stress (Wang et al. 2009). In bivalves, MT plays an important role in the immune system, and its expression is induced by stress, tissue damage, infection, and inflammation (Miles et al. 2000; Wang et al. 2009). Heat shock protein (HSP) is also widely used as a stress marker for bivalves since it plays a diverse role in protecting cells from damage caused by environmental stressors and supports environmental adaptation (Brown et al. 2004; Huang et al. 2014). Finally, when bivalves are exposed to toxic or pathogenic bacteria, their defense mechanisms lead to melanization of epithelial cells in tissues such as the gills and mantle. The synthesized melanin pigment in this process activates a cascade of defense mechanisms to remove ROS and invading pathogens (Vargas-Albores and Barracco 2001; Escobedo-Lozano et al. 2012).

In the study reported here, we used the bay scallop *Argopecten irradians*, which readily accumulates toxins. Bay scallops are economically valuable in various parts of the world and are commercially important shellfish in aquaculture (Bauder et al. 2001; Hégaret and Wikfors 2005; Wang et al. 2009; Chi et al. 2016). They live in shallow water systems (depth < 10 m) and are particularly susceptible to toxic phytoplankton multiplying in the photic zone (Barber and Davis 1997). Very few studies of *Pseudo-nitzschia* have investigated the direct physiological effects of DA on shellfish (Liu et al. 2007; Lelong et al. 2012). Mafra et al. (2009, 2010) reported that the presence of DA in seawater did not have a physiological effect on oysters. Therefore, this study was conducted to investigate the potential effects of DA on the stress response of bay scallops preying on marine diatoms producing DA. The effect of DA on toxicity stress in bay scallops was investigated by exposing the shellfish to three concentrations of DA (20, 40, and 60 ng/ml) for a short time (48 h), following by measuring the changes in the mRNA expression levels of the antioxidant enzymes SOD and CAT and stress-related molecules MT and HSP90 in digestive diverticula. We also measured changes in the levels of antioxidant enzymes and H_2O_2 in hemolymph. We also observed the histological changes in gills exposed directly to toxicity.

Materials and methods

Ethics statement

All experiments were carried out in strict accordance with the guidelines and ethical principles of the Experimental Animal Welfare Ethics Committee of Korea.

Experimental animals

For each experiment, *A. irradians* individuals with a shell length 60 ± 10 mm were purchased from a commercial market in Tongyoung, Korea. Sixty shellfish were allowed to acclimate for 1 week in each of four 400-l circulation filter tanks containing filtered and aerated seawater prior to experiments, with half of the total volume of seawater changed daily. Water temperature and salinity were maintained at 17 °C and 31‰, respectively (JS-WBP-170RP; Johnsam Co., Buchoen, Korea). Scallops were fed a commercial shellfish diet (Instant Algae® Shellfish Diet; Reed Mariculture Inc., Campbell, CA, USA) at a rate of approximately 1.2×10^{10} algae cells per scallop per day prior to experiments.

Exposure experiments

Crystalline DA of ≥ 95% purity (verified by high-performance liquid chromatography) was obtained from Millipore Sigma (St. Louis, MO, USA) and stored at –20 °C prior to use. A total of 240 scallops were randomly divided into control and experimental groups and placed in tanks filled with 30-L seawater. Each control/experimental group consisted of two subgroups of 30 scallops each ($30 \times 2 = 60$ per group for repeat experiments). A DA stock solution (100 µg DA/ml) was prepared by dissolving 5 mg DA in 50 ml deionized water. The experimental groups were treated with 20, 40, or 60 ng/ml DA solution by adding 6, 12, or 18 ml DA stock solution into the respective tank (referred to as the DA 20, DA 40, and DA 60 treatments, respectively); no DA solution was added to the control group (referred to as the DA 0 group). The concentrations were selected on the basis of previous studies reporting on the toxicity of DA accumulation and its effects on juvenile scallops (Liu et al. 2008). Five scallops from each replicate treatment group were randomly collected after 3, 6, 12, 24, 36, and 48 h of exposure, and 1 ml of hemolymph was collected from the adductor muscle

using a 1-ml syringe within 2 min of removing scallops from the tank. This hemolymph was separated by centrifugation at 4 °C, 750 *g* for 3 min. Digestive diverticula, adductor muscle, and mantle tissues were also collected from each scallop and weighed after excess water was removed with absorbent paper. Both samples were then stored at − 80 °C until analysis, for no longer than 1 month.

Domoic acid analysis

Scallops were prepared for DA analysis using a MaxSignal® Domoic Acid Enzyme-Linked Immunosorbent Assay Test kit (BIOO Scientific Corp., Austin, TX, USA), following the manufacturer's instructions. The limit of detection and the limit of quantitation were 0.03 and 0.1 mg/kg, respectively; these values are far lower than the internationally accepted regulatory level (20 mg/kg) of DA. Briefly, DA was extracted from tissues by first homogenizing 0.25 g of tissue in 1 ml of 50% methanol (in water), followed by vortexing for 5 min and then centrifugation at 1500 *g* for 10 min. A 500-µl sample of the supernatant was then transferred to a new tube and heated at 75 °C for 5 min, followed by centrifugation at 1500 *g* for 10 min. Then, a 50-µl sample of the clear supernatant was transferred to a new tube and 950 µl of 1× Sample extraction buffer/methanol (9:1, v/v) was added. For the analysis of hemolymph, 50 µl of hemolymph was mixed well in 950 µl of 1× Sample extraction buffer/methanol (9:1, v/v). Each sample was analyzed for DA.

Total RNA extraction and complementary DNA synthesis

Total RNA was extracted from the digestive diverticula using TRI reagent® (Molecular Research Center, Inc., Cincinnati, OH, USA) according to the manufacturer's instructions. The quality and purity of the RNA were assessed by spectrophotometry by determining the 260:280 ratio (BioDrop, Cambridge, UK). Briefly, total RNA (2 µg) with the oligo-d$(T)_{15}$ anchor and nuclease-free water was heated to 70 °C for 5 min in water at a total volume of ≤ 15 µl to melt the secondary structure within the template, followed by immediate cooling on ice for 1 min, the addition of M-MLV Reaction Buffer (Promega, Madison, WI, USA), 0.5 mM dNTP (Promega), Recombinant RNasin® Ribonuclease Inhibitor (Promega), M-MLV (Promega), and nuclease-free water, and finally incubation for 60 min at 42 °C. Synthesized complementary DNA (cDNA) was stored at − 20 °C until use.

Quantitative PCR

Quantitative PCR (qPCR) was performed to determine the relative expression levels of manganese SOD (MnSOD), CAT, MT, HSP90, and β-actin mRNA using cDNA reverse-transcribed from the total RNA extracted from the digestive diverticula. The primers used for qPCR are shown in Table 1. PCR amplification was conducted using a Bio-Rad CFX96™ Real-time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA) and the iQ™ SYBR Green Supermix (Bio-Rad) under the following conditions: 0.5 µl of cDNA, 0.26 µM of each primer, 0.2 mM dNTP, SYBR Green, and Taq polymerase in buffer (10 mM Tris–HCl [pH 9.0], 50 mM KCl, 1.4 mM $MgCl_2$, and 20 nM fluorescein) to a total volume of 25 µl. The qPCR cycling program consisted of one cycle of initial denaturation at 95 °C for 5 min, 45 cycles of denaturation at 95 °C for 20 s, and annealing at 55 °C for 20 s, followed by a final extension for 10 min at 72 °C. Each experimental group was run in triplicate to ensure consistency. As internal controls, experiments were duplicated with β-actin, and all data are expressed relative to the corresponding β-actin threshold cycle (ΔCt) levels. The calibrated ΔCt value (ΔΔCt) for each sample and internal controls (β-actin) was calculated using the 2ΔΔCt method.

Table 1 Primers used for quantitative PCR amplification

Genes	Primer	DNA sequences
MnSOD (EU137676)	Forward	5′-TAG GGA TTT TGG CTC GTT TG-3′
	Reverse	5′-GTA GGC ATG CTC CCA AAC AT-3′
CAT (GQ265925)	Forward	5′-TAC TGC AAG GCC AAG CTT TT-3′
	Reverse	5′-TTG GGA AAA ATA TGG GGT CA-3′
Metallothionein (EF093795)	Forward	5′-CGG ATG TAA ATG TGC AGG TG-3′
	Reverse	5′-TGC ACG AGT TAG GTC CAG TG-3′
HSP90 (EF532406)	Forward	5′-ATG ACG GAA AGA CCC TTG TG-3′
	Reverse	5′-CAA GGC GGT TGG ATA CAG TT-3′
β-actin (AY335441)	Forward	5′-CAA ACA GCA GCC TCC TCG TCA-3′
	Reverse	5′-CTG GGC ACC TGA ACC TTT CGT T-3′

MnSOD, Manganese superoxide dismutase; CAT, catalase; HSP90, Heat shock protein 90

Analysis of hemolymph parameters

The activities of SOD, CAT, and H_2O_2 in hemolymph were measured using the BM-SOD200, BM-CAT400, and BO-PER500 kits, respectively (Biomax Co., Ltd, Seoul, Korea), according to manufacturer's instructions. One unit of SOD activity was defined as 50% inhibition of the oxidation process (U/ml of hemolymph). One unit of CAT activity was defined as 50% H_2O_2 consumption at 1 min, pH 7.0 (U/ml of hemolymph). SOD, CAT, and H_2O_2 assays were monitored by measuring absorbance using a microplate reader (Victor X3 microplate reader; Perkin Elmer Inc., Waltham, MA, USA) at 450, 560, and 560 nm, respectively.

Gill histology

Gills from the group exposed to 60 ng/ml DA (the highest concentration) were sampled at 3, 24, and 48 h, fixed in Bouin's solution, then dehydrated in a series of increasing concentrations of ethanol solution, clarified in xylene, and embedded in paraffin. The paraffin-embedded gills were then cut into 6-µm-thick sections and stained with hematoxylin and eosin for observation under a light microscope (model Eclipse Ci; Nikon Corp., Tokyo, Japan), and the images were captured using a digital camera (model Eclipse Ci; Nikon Corp.).

Statistical analysis

All data were analyzed using the SPSS statistical package (version 19.0; IBM Corp., Armonk, NY, USA). The normality of the data and the homogeneity of variances were tested using the Shapiro–Wilk test and paired *t* test. A one-way analysis of variance followed by a Tukey's post hoc test were used to compare differences in the data, with significance set at $P < 0.05$. Values were expressed as means ± standard deviation.

Results

Accumulation of domoic acid

None of the bay scallops died during the time they were exposed to DA. At the pre-specified time points, scalop were randomly sampled, and DA concentrations were measured in the digestive diverticula, adductor muscle, and mantle. Overall, the highest concentration of DA in the digestive diverticula was observed at 48 h after exposure to 60 ng/ml DA (digestive diverticula 2.61 ± 0.16 ng/ml; adductor muscle 0.27 ± 0.02 ng/ml; mantle 0.30 ± 0.02 ng/ml) (see Fig. 1 and Electronic Supplementary Material 1). In addition, at all sampling times, the concentration of DA in digestive diverticula was highest following exposure to 60 ng DA/ml, with the highest DA concentration observed in bay scallops exposed to this concentration for 48 h (2.61 ± 0.16 ng/ml), followed in order of decreasing DA concentration in the digestive diverticula with exposure to 40 and 20 ng DA/ml (1.72 ± 0.10 and 1.02 ± 0.06 ng/ml, respectively) at all sampling times ($P < 0.05$; Fig. 1a). The highest concentration in hemolymph was following exposure to 60 ng/ml (1.44 ± 0.10 ng/ml), with the highest level observed at 48 h, followed by exposure to 40 and 20 ng/ml (1.25 ± 0.08 ng/ml and 0.80 ± 0.06 ng/ml) ($P < 0.05$; Fig. 1b).

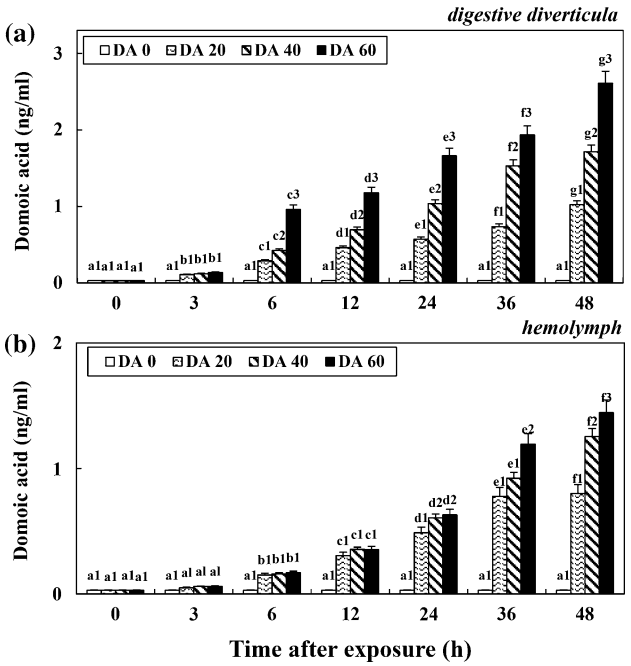

Fig. 1 Changes in domoic acid (DA) concentration in digestive diverticula (**a**) and hemolymph (**b**) of bay scallops (*Argopecten irradians*) exposed to DA for up to 48 h. Different lowercase letters indicate a signficant difference ($P < 0.05$) among scallops exposed for different durations but at the same DA concentration. Different numbers indicate significant differences ($P < 0.05$) among the measured DA concentration at the same exposure time All values represent mean ± standard deviation (SD) ($n = 5$)

Expression of MnSOD and CAT mRNA and MnSOD and CAT enzymatic activities

The expression levels of MnSOD and CAT mRNA and the enzymatic activities of MnSOD and CAT increased significantly ($P < 0.05$) with exposure to increasing DA concentrations in both the digestive diverticula and hemolymph (Figs. 2, 3). mRNA expressions and enzymatic activities in all treatment groups were higher at each sampling time compared to the respective controls, with the highest expression values observed at 24 h (MnSOD mRNA 12.67 ± 0.76 [relative to β-actin]; SOD activity 30.00 ± 2.50 U/ml; CAT

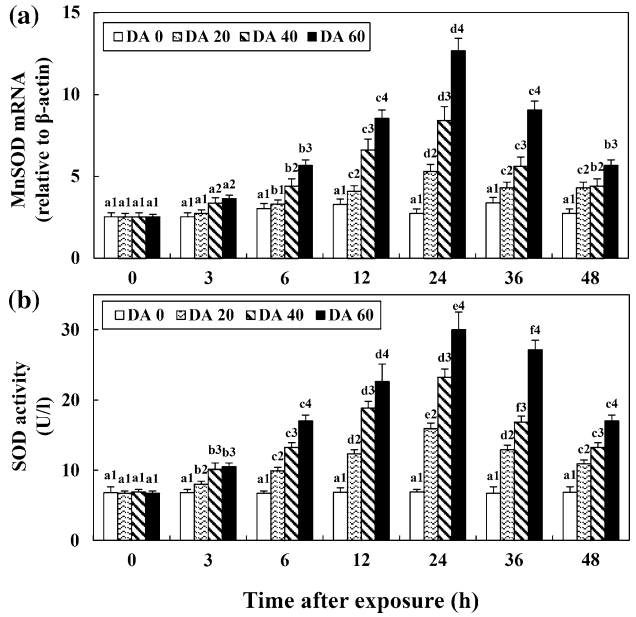

Fig. 2 Changes in expression of manganese superoxide dismutase (*MnSOD*) mRNA (relative to β-actin) in the digestive diverticula (**a**) and in superoxide dismutase (*SOD*) enzymatic activity in the hemolymph (**b**) of bay scallops exposed to DA for up to 48 h. Different lowercase letters indicate significant differences ($P<0.05$) among scallops exposed for different durations within the same DA concentration. Numbers indicate significant differences ($P<0.05$) among scallops exposed to different DA concentration for the same duration. All values represent the mean ± SD ($n=5$)

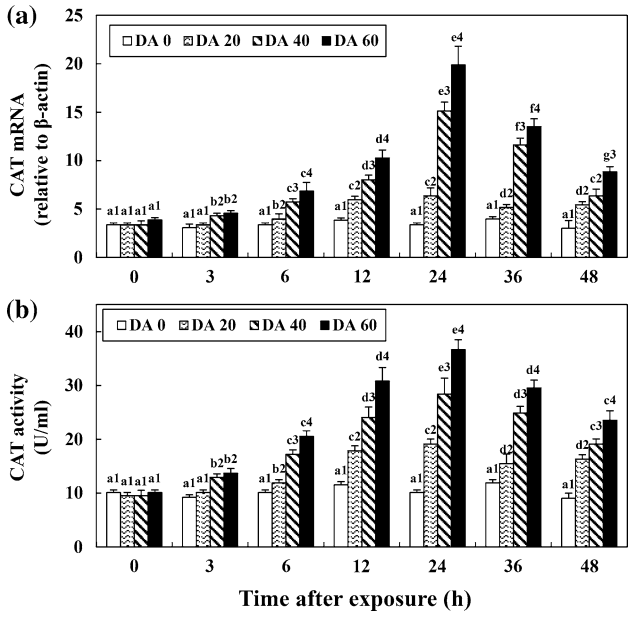

Fig. 3 Changes in expression of catalase (*CAT*) mRNA in the digestive diverticula (**a**) and in CAT enzymatic activity in the hemolymph (**b**) of bay scallops exposed to DA for up to 48 h. Different lowercase letters indicate significant differences ($P<0.05$) among scallops exposed for different durations within the same DA concentration. Numbers indicate significant differences ($P<0.05$) among scallops exposed to different DA concentration for the same duration. All values represent the mean ± SD ($n=5$)

mRNA 19.89 ± 1.93 [relative to β-actin]; CAT enzymatic activity 36.67 ± 1.83 U/ml). All measurements were significantly higher in the bay scallops treated with 60 ng/ml than in other treatment groups.

H_2O_2 concentration

The concentration of H_2O_2 in hemolymph significantly increased with increasing DA dose (Fig. 4) ($P<0.05$), with the H_2O_2 concentration higher in all treatment groups than in the respective controls at each sampling time. The highest concentrations observed at 24 h. H_2O_2 concentrations were significantly higher in the group treated with 60 ng/ml than in other groups (at 24 h, DA 60: 42.76 ± 1.83 μM; $P<0.05$).

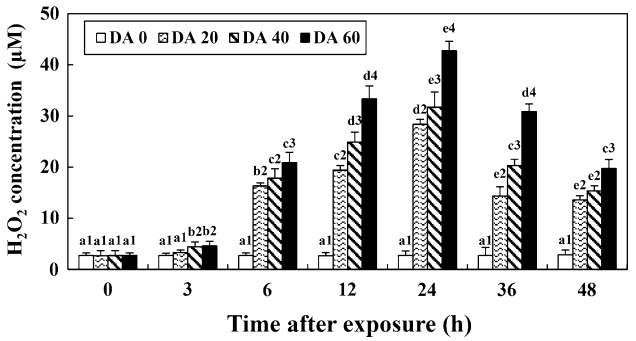

Fig. 4 Concentration of hydrogen peroxide (H_2O_2) in the hemolymph of bay scallops exposed to DA for up to 48 h. Different lowercase letters indicate significant differences ($P<0.05$) among scallops exposed for different durations within the same DA concentration. Numbers indicate significant differences ($P<0.05$) among scallops exposed to different DA concentration for the same duration. All values represent the mean ± SD ($n=5$)

Expression of MT mRNA

The expression of MT mRNA significantly increased with increasing DA dose (Fig. 5a), with expression levels significantly higher in the group treated with DA at 60 ng/ml than in other groups. The expressions of MT mRNAs were higher in all treatment groups than in the respective controls at each sampling time, with the highest expression values observed at 12 and 24 h (DA 60: 6.43 ± 0.58 and 4.64 ± 0.42, respectively; $P<0.05$).

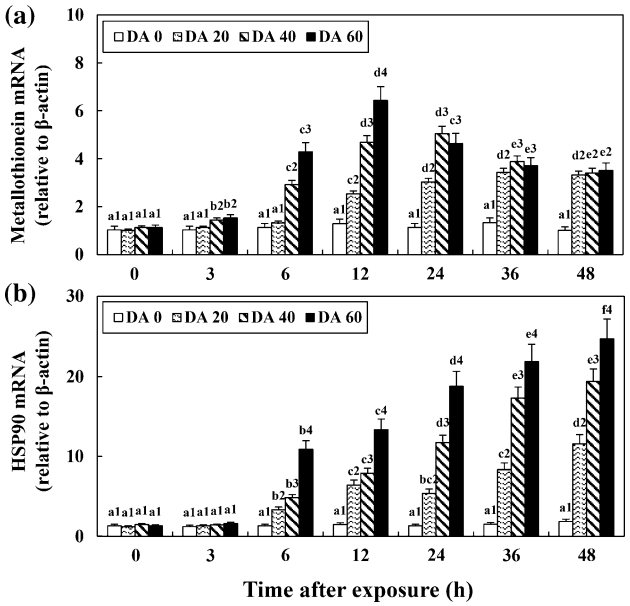

Fig. 5 Changes in the expression levels of metallothionein mRNA (**a**) and heat shock protein 90 (*HSP90*) mRNA (**b**) in the digestive diverticula of bay scallops exposed to DA for up to 48 h. Different lowercase letters indicate significant differences ($P < 0.05$) among scallops exposed for different durations within the same DA concentration. Numbers indicate significant differences ($P < 0.05$) among scallops exposed to different DA concentration for the same duration. All values represent the mean ± SD ($n = 5$)

Expression of HSP90 mRNA

There was no signficant increase in the expression of HSP90 mRNA in the digestive diverticula of bay scallops in each treatment group at 3 h (Fig. 5b). After 3 h, HSP mRNA expression in the group treated with DA at 60 ng/ml was significantly higher than that in the other groups. All treatment groups showed a gradual increase in HSP90 mRNA with increasing exposure time. After 6 h, HSP90 mRNA was significantly upregulated, to a maximum at 48 h (DA 60; 24.72 ± 2.46; $P < 0.05$).

Gill histology

There were visible differences in the mean darkness of melanin granules between control gills and gills from individuals exposed to DA at 60 ng/ml (Fig. 6). Epithelial melanization of gills took place under exposure to DA at 60 ng/ml for 48 h ($P < 0.05$). The gill area became more melanized with increasing exposure time.

Discussion

In vertebrates, the ingestion of DA-contaminated shellfish can lead to excitotoxic responses in the central nervous system and potentially to memory loss (Zabaglo et al. 2016). It

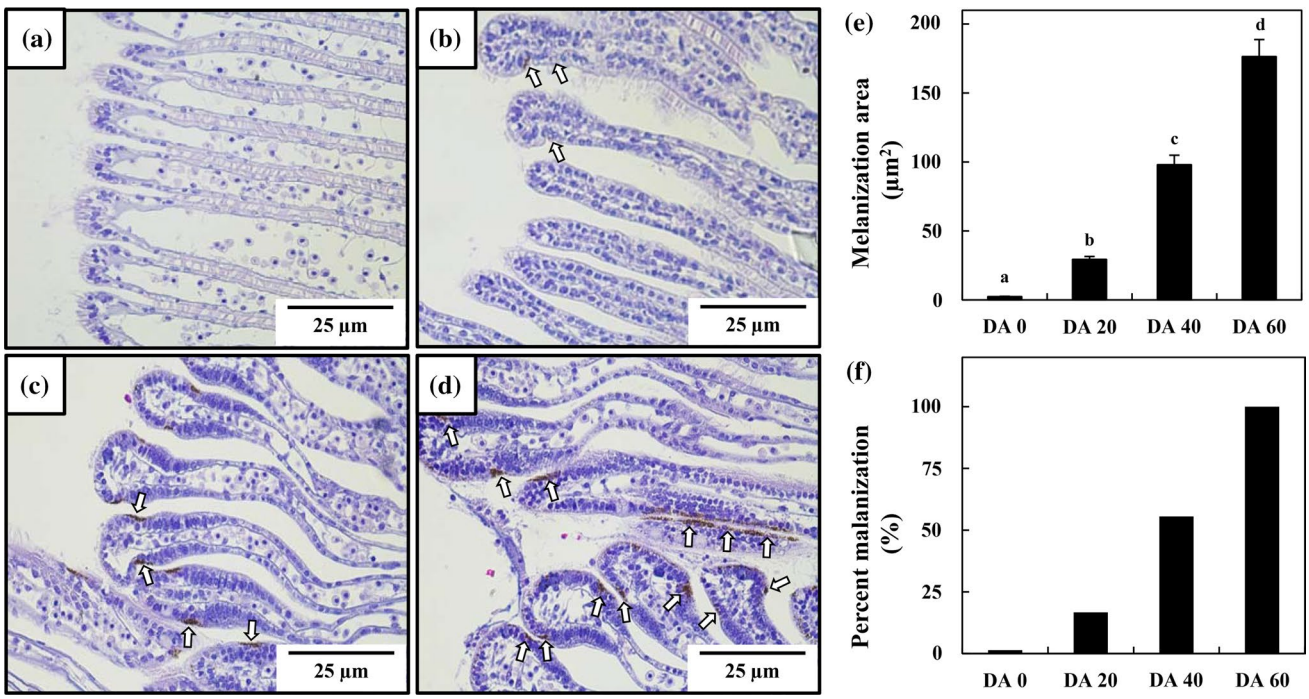

Fig. 6 Gill histology. a–d Cross section of gills of control bay scallops (**a**) and bay scallops exposed to DA for 3 h (**b**), 24 h (**c**), and 48 h (**d**). White arrows indicate gills with melanization. Scale bar: 25 μm. **e** Measured area of epithelial melanization in the gills. **f** Melanization area of gills exposed to each concentration divided by the melanization area of gills exposed to DA 60

has been well established that DA poses a threat to human health, but the relevance of shellfish responses to DA have not been demonstrated. In this study, we investigated indicators of oxidative stress and histological changes to gills in bay scallops exposed to various concentrations of DA with the aim to clarify any potential effects on humans.

We observed that the accumulation of DA in the digestive diverticula and hemolymph increased with increasing duration of DA exposure and increasing DA concentration. Mafra et al. (2009, 2010) reported that the presence of DA played no role in oysters and concluded that oysters do not accumulate DA in tissues when exposed directly to DA in their environment. However, an earlier study confirmed that DA does accumulate in the liver and pancreas of sea scallops (Douglas et al. 1997), suggesting that DA may accumulate in bay scallops both directly from the seawater as well as from feeding on toxic *Pseudo-nitzschia*. These results also suggest that shellfish predators in the food web are at greater risk when blooms of *Pseudo-nitzschia* are sustained.

Many previous studies have focused on the morphology, distribution, and toxicity of DA-producing algae species, including *Pseudo-nitzschia* spp., *Nitzschia bizertensisand*, and *Nitzschia navis-varingica* (Bauder et al. 2001; Trainer et al. 2012). Therefore, the aim of our study was to confirm that it is DA, not algae species that produce DA, which directly generates oxidative stress in bay scallops. We measured the antioxidant mechanisms of shellfish for coping with these toxic stresses and noted that higher DA concentrations were associated with higher H_2O_2 levels, as well as with a higher mRNA expression and activity of antioxidant enzymes and that these higher levels tended to increase until 24 h after exposure to each DA concentration, following which time they tended to decrease. In previous studies, the mussel *Mytilus galloprovincialis* exposed to *Alexandrium tamarense*, which induces paralytic shellfish poisoning, showed increasing SOD and CAT activities in the digestive gland and muscle for up to 72 h and 96 h after exposure, respectively, and then decreasing activities after these time points (Qiu et al. 2013). These results suggest that the expression of CAT mRNA and enzymatic activity of CAT increase protectively to remove H_2O_2 produced under oxidative stress due to toxin exposure induced by algal toxicity, but that after 24 h of sustained exposure, molluscan defense mechanisms may become overwhelmed as algal toxins accumulate.

In our study, MT mRNA expression followed a similar pattern as that for CAT mRNA, increasing in the first 12–24 h of DA exposure, but decreasing thereafter. Chi et al. (2016) reported that when *Argopecten irradians* were exposed to okadaic acid (OA) phycotoxin, MT mRNA expression increased with increasing concentrations of OA but fell over time. Based on these findings, it appears that tissue damage and oxidative stress induced by DA is mitigated by MT, but as with SOD and CAT, sustained exposure exceeds defense capacity.

Similarly, we observed that HSP90 increased with the concentration of DA and duration of DA exposure. Manfrin et al. (2010) reported that the mussel *Mytilus galloprovincialis* showed a gradual increase in HSP90 expression when exposed to OA for 3 days, and Chi et al. (2016) reported that *A. irradians* exposed to OA showed significantly increased HSP90 for up to 48 h at both low and high concentrations of OA. In both of these species, HSP is likely continuously produced to counteract the protein damage that results as the antioxidant system fails under toxin exposure.

We also observed that melanization also gradually increased with increasing duration of DA exposure. Estrada et al. (2007) found that when the giant lions-paw scallop *Nodipecten subnodosus* was exposed to *Gymnodinium catenatum*, a toxic dinoflagellate, shell movement was reduced and melanization progressed in the gills. A comparable observation was made in the Pacific calico scallop *Argopecten ventricosus* exposed to the same algae (Escobedo-Lozano et al. 2012). The results of this study indicate that the ROS produced by DA increased with increasing exposed concentration of DA and that the defense system became increasingly activated. As a result, the range of melanization in the gills was widened.

In summary, DA in the seawater can be accumulated in the tissues of bay scallops. Our results indicate that exposure to DA toxic concentrations > 20 ng/ml for at least 12 h may lead to a decrease in the activity of defense mechanisms in bay scallops. Antioxidants and other defense systems are activated in bay scallops to remove ROS produced with exposure to DA but may become exhausted under sustained exposure to the levels studied here. Further research should be conducted to investigate the pathways through which DA directly affects scallops. Additionally, future studies could examine the means of supporting these physiological responses in farmed scallops under stress from HABs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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