

Article

Antioxidant Responses of the Pacific Abalone *Haliotis discus hannai* to Turbidity Changes

Jin Ah Song ¹, Dong Mun Choi ¹, Yun-Hwan Jung ², Heung-Sik Park ³, Taihun Kim ³, Seog-Il Jang ² and Dae-Won Lee ^{1,*}

¹ Marine Biotechnology and Bioresource Research Department, Korea Institute of Ocean Science and Technology, Busan 49111, Republic of Korea; jinah@kiost.ac.kr (J.A.S.); dmchoi@kiost.ac.kr (D.M.C.)

² Tongyeong Maritime Test & Evaluation Station, Korea Institute of Ocean Science and Technology, Tongyeong 53087, Republic of Korea; aries0324@kiost.ac.kr (Y.-H.J.)

³ Jeju Research Institute, Korea Institute of Ocean Science and Technology, Jeju 63349, Republic of Korea; tk2020@kiost.ac.kr (T.K.)

* Correspondence: dw0130@kiost.ac.kr; Tel.: +82-51-664-3323; Fax: +82-51-955-3981

Abstract

The increasing use of water-based drilling muds in offshore oil and gas operations has raised concerns about potential ecological risks of their primary components, such as bentonite, on marine organisms. To date, the biological effects of bentonite on benthic species remain poorly understood. This study aimed to evaluate the physiological and oxidative stress responses of Pacific abalone (*Haliotis discus hannai*) exposed to varying concentrations (20–3000 mg/L) of bentonite over a 10-day period. Short-term exposure (up to 7 days) to bentonite did not result in significant mortality across treatment groups; however, partial mortality was observed in the highest concentration group (3000 mg/L) on day 8. Biochemical analyses revealed elevated levels of hydrogen peroxide and malondialdehyde, particularly in higher concentration groups, indicating oxidative stress. Antioxidant enzyme activities showed concentration- and time-dependent changes, with early activation followed by suppression under prolonged exposure. Total antioxidant capacity also declined over time in high-concentration groups. These findings indicate that while bentonite may not be acutely lethal to abalone, it can trigger sublethal oxidative stress responses, particularly under chronic exposure conditions, underscoring the importance of evaluating long-term physiological impacts of suspended drilling particulates and the need for research on a wider range of marine species.

Keywords: antioxidant activity; bentonite; *Haliotis discus hannai*; water-based drilling muds; drilling particulates

Key Contribution: Short-term exposure to bentonite induces oxidative stress without causing mortality, whereas long-term exposure may lead to death due to sustained oxidative stress.



Academic Editor: Jack Frankel

Received: 5 August 2025

Revised: 29 August 2025

Accepted: 4 September 2025

Published: 5 September 2025

Citation: Song, J.A.; Choi, D.M.; Jung, Y.-H.; Park, H.-S.; Kim, T.; Jang, S.-I.; Lee, D.-W. Antioxidant Responses of the Pacific Abalone *Haliotis discus hannai* to Turbidity Changes. *Fishes* **2025**, *10*, 455. <https://doi.org/10.3390/fishes10090455>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Since the early 20th century, the discharge of drill cuttings generated from nonaqueous drilling fluids (NADFs) into marine environments has been effectively prohibited due to stringent environmental regulations. This restriction stems from the toxicological concerns and the environmental persistence of base oils commonly used in NADFs. Operators utilizing NADFs are currently required to either wash cuttings to reduce base oil content

below 1%—a process that is both technically challenging and costly—or to transport the cuttings onshore for disposal or reinject them into subsurface formations. In contrast, drill cuttings produced from water-based muds (WBM) are generally considered to pose minimal environmental risks and are therefore permitted for offshore discharge. WBMs have undergone significant improvements in recent years, with modifications aimed at enhancing their lubricity, thermal stability, and environmental compatibility. These advancements include the incorporation of environmentally benign additives such as synthetic polymers (e.g., partially hydrolyzed polyacrylamide), biodegradable lubricants, and low-toxicity shale inhibitors like potassium chloride (KCl) or glycols. Furthermore, fluid loss control agents (e.g., starch, cellulose derivatives), viscosifiers (e.g., bentonite, xanthan gum), and pH control agents (e.g., lime, caustic soda) are carefully selected to minimize marine toxicity. Most components are either non-toxic or used at concentrations low enough to exert negligible toxicological effects on marine life [1]. However, despite these improvements, certain constituents—such as biocides or high salt concentrations—may still exert adverse effects on specific marine species [2].

Bentonite is one of the primary components of water-based drilling muds (WBMs) and plays a crucial role in maintaining fluid viscosity and facilitating the transport of drill cuttings. This material forms a thixotropic gel structure that liquefies under mechanical agitation caused by the rotation of the drill string and bit, allowing the slurry to flow efficiently through the main and annular wellbores. When drilling operations are paused, bentonite reverts to its gelled state, suspending drill cuttings and weighting materials, as well as preventing their rapid sedimentation. Simultaneously, it forms a filter cake along the borehole wall, minimizing fluid infiltration into permeable rock formations. These characteristics are essential for effective removal of cuttings and borehole stability [3].

Exposure to suspended particles, including bentonite—a major component of water-based drilling muds—has been shown to adversely affect suspension-feeding bivalves. For example, a comparative study demonstrated that exposure to bentonite particles can affect filtration activity and survival in suspension-feeding bivalves [4]. Additionally, Northwest Atlantic scallops (*Placopecten magellanicus*) exhibited low tolerance to suspended bentonite, indicating a possible adverse effect on this species' physiological functions [5].

Despite its widespread use, there is a notable lack of research on the potential impacts of bentonite on benthic marine organisms, particularly with regard to mollusks. While previous research has primarily focused on the effects of modified clays on aquaculture species, including finfish [6,7] and bivalves [8], little is known about the responses of abalone to such materials.

Exposure to environmental pollutants can lead to oxidative stress in marine organisms by disrupting the balance of reactive oxygen species (ROS) [9–11]. To evaluate such responses, antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) are widely used as biomarkers of oxidative stress. These enzymes play key roles in cellular defense mechanisms and serve as effective indicators of physiological changes under environmental stress [12]. In this study, we used SOD, CAT, and GST activities to assess the potential oxidative impact of bentonite exposure on the antioxidant defense system in abalone.

The Pacific abalone (*Haliotis discus hannai*) is a commercially and ecologically important marine gastropod widely cultivated and harvested along the coasts of East Asia, especially in South Korea, China, and Japan [13,14]. It plays a key role in coastal ecosystems as an algal grazer and is also highly valued in aquaculture due to its high market demand and nutritional value in human diets, particularly in East Asian countries. In addition to its economic and ecological significance, the consumption of *H. discus hannai* has been associated with anti-inflammatory benefits, partly attributed to its phenoloxidase content,

which plays a key role in innate immune responses and oxidative stress regulation in mollusks [15,16]. These coastal zones also overlap with regions of active offshore oil and gas exploration, where water-based drilling fluids (WBM), including bentonite, are frequently discharged into the marine environment [17,18]. As a benthic and relatively sedentary species, abalone is particularly susceptible to the accumulation of drilling-derived particulates and the resulting physiological stress. Moreover, its filter-feeding behavior and close contact with the sediment make it a suitable sentinel species for assessing sublethal impacts of drilling discharges. Despite this, few studies have focused on the oxidative stress response of abalone to WBM exposure. Therefore, this study aims to evaluate the potential oxidative effects of bentonite on *H. discus hannai*, providing insight into the environmental risks of offshore drilling activities in habitats critical to this species.

This study aimed to investigate the effects of bentonite exposure on the survival and antioxidant responses of Pacific abalone *Haliotis discus hannai*, a representative commercial benthic species.

2. Materials and Methods

2.1. Abalones

Specimens of Pacific abalone *Haliotis discus hannai* for the experiments were collected in a cultivation facility of Tongyeong, Gyeongsangnam-do, South Korea (shell length 31.02 ± 3.99 mm, shell weight 3.58 ± 1.02 g). The abalones were transported to the laboratory in Busan, where all experiments were conducted. They were maintained in 100 L rectangular acrylic tanks filled with filtered natural seawater (1 μ m filter), continuously aerated to maintain dissolved oxygen (DO) levels above 6.5 mg/L. The water temperature was controlled at 22.0 ± 0.6 °C using an automatic thermostat system, and pH was maintained at 8.1 ± 0.2 . A 12 h light: 12 h dark photoperiod was maintained under low-intensity fluorescent lighting (approx. 100 lx). The stocking density was approximately 30 individuals per 20 L tank, and partial water changes (50%) were performed every day. The abalones were allowed to acclimate for 2 weeks under these conditions and were fed daily with fresh sea mustard (*Undaria pinnatifida*) throughout the acclimation period.

2.2. Suspended Sediment

The bentonite used in our experiments was commercially sourced (285234; Sigma, Saint Louis, MO, USA), and its physicochemical properties are as follows: mean particle size of ~10 μ m, cation exchange capacity of 70–80 meq/100 g, and pH of ~10.5 in aqueous suspension. The use of bentonite as a representative component allows for the controlled evaluation of its specific effects on marine organisms without the confounding influence of other substances.

In Busan, the maximum concentration of suspended sediments in bottom waters near areas of intensive coastal development, such as landfills and industrial ports, was reported to be 92.6 mg/L [19]. This value was derived from in situ water sampling conducted at approximately 1 m above the seafloor using a Niskin sampler, followed by filtration and gravimetric analysis in accordance with standard methods [20].

For toxicity reference, Yoon and Park [21] reported a 7-day LC₅₀ value of 1887 mg/L and a lowest observed effect concentration (LOEC) of 500 mg/L for *Haliotis discus hannai* based on survival and glycogen depletion. However, as this previous study focused only on organism-level endpoints, our study aimed to examine sublethal physiological responses, particularly oxidative stress biomarkers. Therefore, we selected a broader range of experimental concentrations including values below and above the LOEC: 0, 20, 200, 500, 1000, 2000, and 3000 mg/L.

2.3. Experimental Design

The suspended sediment exposure experiment was conducted using a 20 L conical polyethylene water tank placed inside a larger temperature-controlled water bath to maintain a stable water temperature throughout the experiment (Figure 1). To ensure a uniform suspension of particles, an aeration device was installed at the base of each conical tank. In addition, to prevent abalone from escaping due to their movement along the tank wall, the upper opening of each tank was securely covered with a mesh bag. This setup allowed for consistent thermal and physical conditions during the exposure period, minimizing external disturbances.

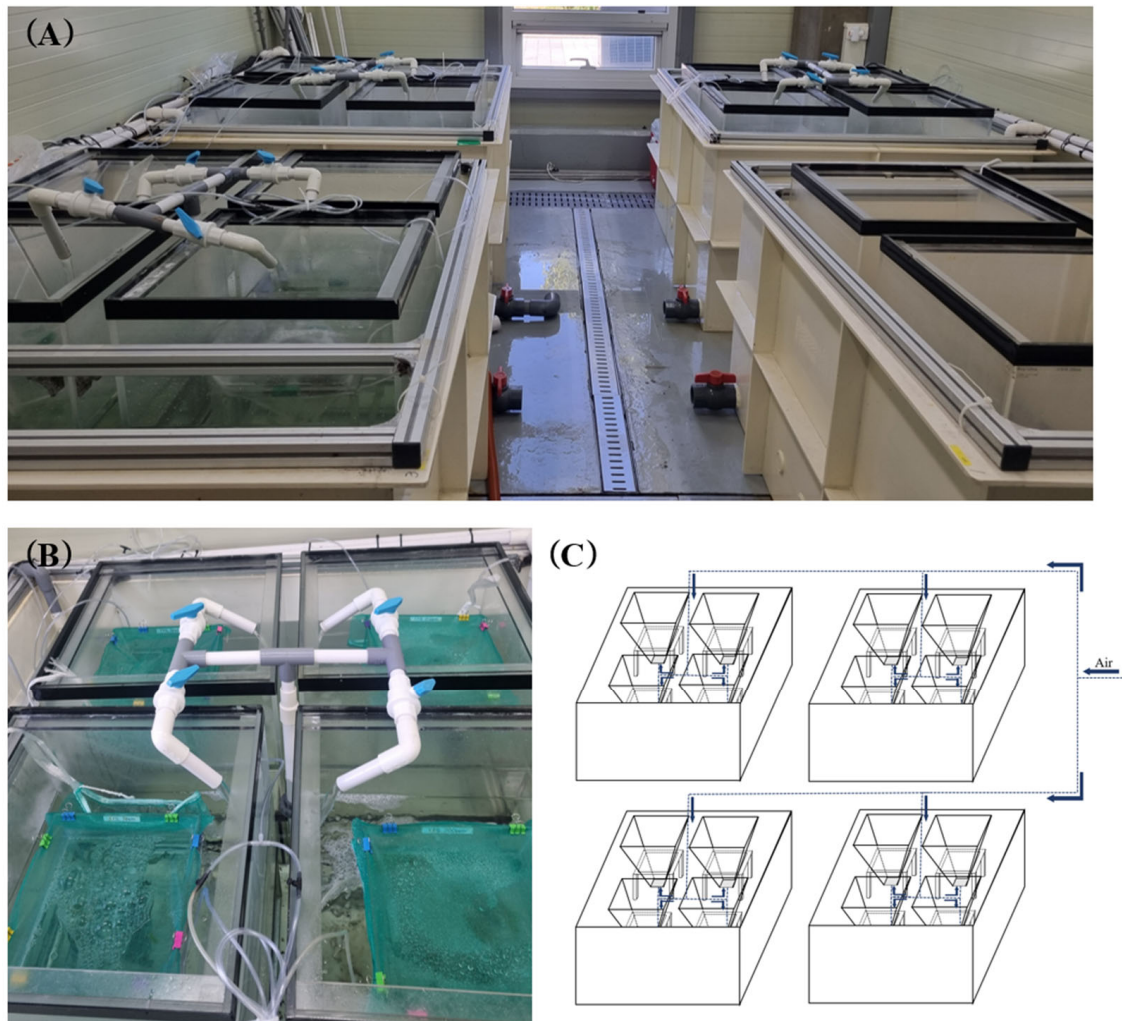


Figure 1. (A) Photograph of the full experimental tank setup used in this study. (B) Photograph of a single temperature-controlled water bath containing a conical experimental tank. (C) Schematic diagram of the experimental tank used for suspended sediment exposure experiments. Arrows indicate the direction of aeration flow.

Throughout the experiment, key water quality parameters were monitored daily, including temperature, dissolved oxygen (DO), pH, nitrate (NO_3^-), and nitrite (NO_2^-). Temperature was maintained at 22.0 ± 0.6 °C using an automatic temperature controller (JEIO TECH TH-T, Incheon, Republic of Korea). DO and pH were measured using a YSI ProDSS multiparameter water quality meter (YSI Inc., Yellow Spring, OH, USA). Nitrate and nitrite concentrations were analyzed using a colorimetric method with a DR3900 spectrophotometer (Hach Co., Loveland, CO, USA), following the manufacturer's protocol.

Turbidity of the experimental water was monitored using a turbidimeter (Hach 2100Q Portable Turbidimeter, Hach Co., Loveland, CO, USA) to ensure consistent suspended particulate concentrations across treatments.

Abalones were randomly selected and transferred to 20 L tanks at a density of 30 abalones per tank. The suspended sediments were diluted according to the experimental concentration (0, 20, 200, 500, 1000, 2000, or 3000 mg/L). For each concentration group, three parallel replicate tanks were prepared. A stock solution of bentonite (6000 mg/L) was prepared by vigorously mixing the dry powder with filtered seawater using a magnetic stirrer. This solution was then diluted to the target concentrations with filtered seawater and gently stirred to maintain suspension.

During the period, abalones were fed daily with fresh sea mustard (*Undaria pinnatifida*) to maintain their normal physiological condition. To prevent the accumulation of organic matter from the feed, which could potentially influence water quality and interfere with suspended sediment concentrations, the seawater in each tank was replaced daily with freshly prepared bentonite solutions at the respective treatment concentrations. The survival of the abalone was observed daily. Mortality was defined as the complete lack of movement and no muscular response to tactile stimulation, accompanied by relaxed musculature and gaping shells. Five abalones were randomly selected at 1, 4, 7 and 10 days after exposure, and abalones in each sample were anesthetized using MS-222 (A5040; Sigma, Saint Louis, MO, USA). Hepatopancreas tissue samples collected from the abalones on days 1, 4, 7, and 10, and immediately stored in a -80°C refrigerator until further analysis could be carried out.

2.4. H_2O_2 Activity, Total Antioxidant Capacity (TAC) Assays, and MDA Contents

For each treatment group, hepatopancreas tissues were individually collected from each abalone and were homogenized in $1\times$ phosphate-buffered saline (PBS). The homogenates were centrifuged at $5000\times g$ for 10 min at 4°C , after which the supernatant was removed, and the remaining pellet was used for analysis. Prior to analysis, total protein concentration in each sample was determined using the Bradford method (Bio-Rad, Hercules, CA, USA), with bovine serum albumin (BSA) as the standard. The average total protein concentration across all groups ranged from 1.25 ± 0.18 mg/mL to 1.49 ± 0.21 mg/mL, and all assay results were normalized to protein content.

H_2O_2 activity, TAC, and MDA contents, respectively, were measured using BO-PER500, BO-TAC-200, and BO-TBR-200 assay kits (BIOMAX Co., Ltd., Seoul, Republic of Korea), according to the manufacturer's instructions. TAC was measured based on the Trolox equivalent antioxidant capacity and the assay is based on the reduction of copper (II) to copper (I) by antioxidants, which reflects the level of ROS. MDA reacts with thiobarbituric acid (TBA) to form an MDA-TBA adduct, and the degree of lipid peroxidation can be determined by measuring this. The H_2O_2 , TAC and MDA assays were evaluated by measuring absorbance at 560 nm, 450 nm and 532 nm using a SpectraMax[®] iD5 Multi-Mode Microplate Reader (Molecular Devices, San Jose, CA, USA).

2.5. Antioxidant Enzyme Activities

Individual hepatopancreas samples were homogenized in $1\times$ PBS and centrifuged ($5000\times g$, 10 min, 4°C); the resulting pellet was used for biochemical assays. Total protein was measured using the Bradford method (Bio-Rad) with BSA as a standard, yielding concentrations between 1.25 ± 0.18 mg/mL to 1.49 ± 0.21 mg/mL, and all data were normalized per mg of protein. The activities of SOD and CAT were measured using the BM-SOD200 and BM-CAT400 kits, respectively (Biomax), according to the 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% H₂O₂ consumption at 1 min, pH 7.0 (U/mL of hemolymph). SOD and CAT assays were evaluated by measuring absorbance using a SpectraMax® iD5 Multi-Mode Microplate Reader (Molecular Devices) at 450 nm and 560 nm, respectively.

The GST activity was measured using a GST cellular activity assay kit (Sigma–Aldrich Co., St. Louis, MO, USA). Briefly, samples were homogenized in sample buffer (2 mM Tris–HCl, containing 20% glycerol, 2 mM mercaptoethanol, and 0.5 mM PMSF [pH 8]). The homogenates were centrifuged at 13,000× *g* for 20 min at 4 °C. The cytosolic fraction containing the enzyme was collected for an enzymatic assay with 1-chloro-2,4-dinitrobenzene (CDNB, the extinction coefficient for which is 9.6 mM·cm^{−1}) as a substrate. The enzymatic assay was used to measure the conjugation of CDNB and glutathione (GSH) at 340 nm using a spectrophotometer at 25 °C.

2.6. Statistical Analysis

All data were analyzed using SPSS (version 21.0; SPSS Inc., Chicaco, IL, USA). For bentonite concentration and exposure day, the data of individuals used in each experiment were compared using one-way ANOVA, followed by Duncan’s multiple comparison post hoc test. To assess potential interactions between light intensity and photoperiod, two-way ANOVA was employed. Differences were considered statistically significant at *p* < 0.05. All results are presented as means ± standard error (SE).

3. Results

3.1. Survival Rate

Survival rates of abalones were assessed after 10-day exposure to varying concentrations of bentonite. Survival rates for each group are summarized in Table 1. Each treatment group was composed of three replicate tanks.

Table 1. Survival rate of *H. discus hannai* subjected to the following experimental exposure: 0, 20, 200, 500, 1000, 2000, or 3000 mg/L of bentonite clay for 10 d.

Bentonite (mg/L)	Survival Rate (%)									
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
20	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	96. ± 4.2 *
200	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	99.7 ± 0.3 *	99.7 ± 0.3 *	93.1 ± 3.0 **
500	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	96.6 ± 2.4 *	96.0 ± 4.0 *	96.0 ± 4.0 *
1000	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	96.5 ± 1.2 *	96.5 ± 1.2 *	95.8 ± 2.0 *
2000	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	93.3 ± 3.3 **	93.3 ± 4.2 **	92.2 ± 4.5 **
3000	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	90.0 ± 1.1 **	90.0 ± 4.2 **	90.0 ± 4.2 **

* Note: values not sharing a common superscript, *p* < 0.05 (*), *p* < 0.01 (**).

Overall, survival decreased with increasing bentonite concentration, although the 200 mg/L group exhibited no mortality. No mortality occurred in any group during the first 7 days. However, beginning on day 8, mortality was observed in groups exposed to 500 mg/L or higher. In the 3000 mg/L group, a mortality rate of 10 ± 5.8% was recorded by day 10.

3.2. H₂O₂ Activity and MDA Content

Exposure of *H. discus hannai* to suspended bentonite particles induced significant changes in oxidative stress markers, particularly H₂O₂ and MDA levels. H₂O₂ concentrations increased in all treatment groups from day 1 of exposure. In lower concentration groups (<500 mg/L), H₂O₂ levels slightly declined after day 4, whereas in higher concentration groups (≥1000 mg/L), levels continued to rise throughout the 10-day exposure period. The highest H₂O₂ concentrations were recorded on day 10 in the 2000 mg/L

($44.74 \pm 4.21 \mu\text{M}$) and 3000 mg/L ($50.69 \pm 4.21 \mu\text{M}$) groups, with significant differences compared to the control ($p < 0.05$; Figure 2A). Two-way ANOVA revealed statistically significant main effects of both exposure time ($F = 34.215$, $p = 0.045$) and bentonite concentration ($F = 3.495$, $p = 0.039$) on H_2O_2 levels, while the interaction between time and concentration was not significant ($F = 0.784$, $p = 0.195$), indicating independent influences of these variables.

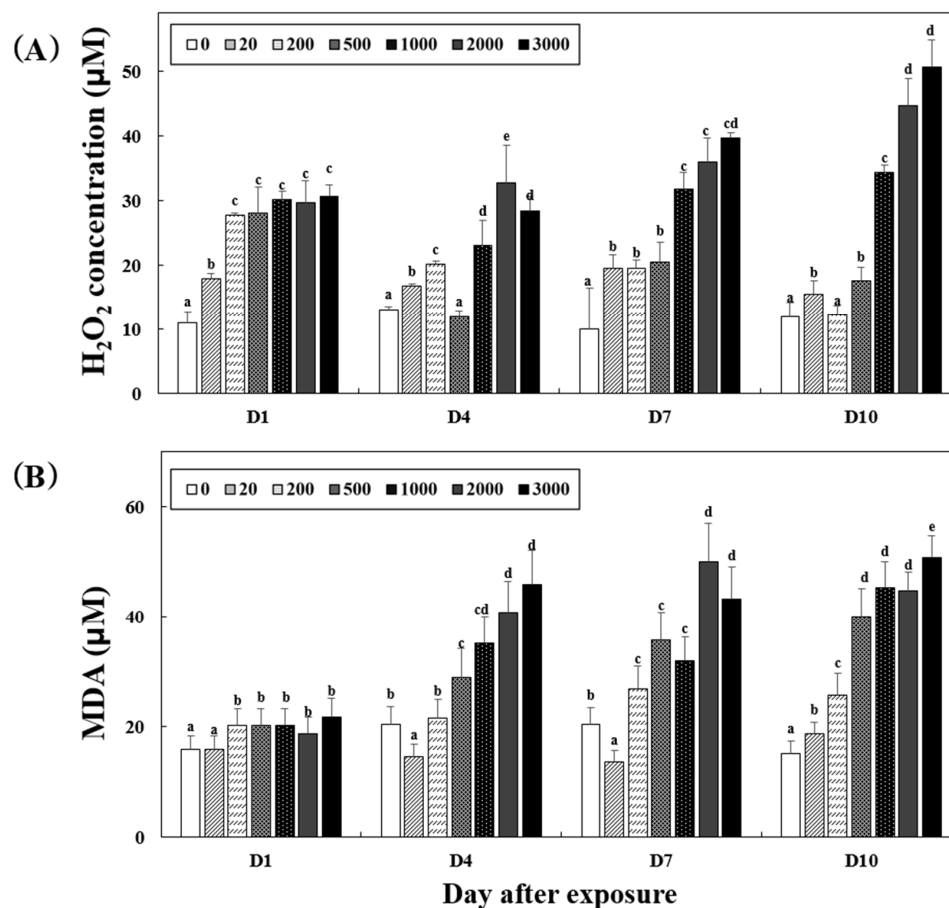


Figure 2. Changes in the H_2O_2 concentration (A) and malondialdehyde (MDA) content (B) in the hepatopancreas of *H. discus hannai* subjected to the following experimental exposure: 0, 20, 200, 500, 1000, 2000, or 3000 mg/L of bentonite clay for 10 d. Different lowercase letters indicate significant differences between different concentrations within the same exposure day ($p < 0.05$). All values are means $\pm$ standard error (SE; $n = 3$).

MDA content, an indicator of lipid peroxidation, also increased significantly from day 1 in all groups except the 20 mg/L group ($p < 0.05$). MDA levels showed a concentration-dependent increase over time, with the highest value observed on day 10 in the 3000 mg/L group ($50.79 \pm 4.00 \mu\text{M}$) (Figure 2B). Consistent with H_2O_2 trends, significant effects of time ($F = 30.144$, $p = 0.039$) and bentonite concentration ($F = 1.352$, $p = 0.044$), with no significant interaction ($F = 0.894$, $p = 0.395$).

3.3. Antioxidant Enzymatic Activities

The activities of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in response to bentonite exposure are presented in Figure 3. Both enzymes exhibited a significant increase in activity during the initial 7 days of exposure, with enzyme activity positively correlated with bentonite concentration ($p < 0.05$). However, divergent trends emerged by day 10. In the case of SOD, activity decreased markedly in the high-concentration groups (≥ 1000 mg/L), potentially indicating enzyme inhibition or exhaustion

of antioxidant defenses due to sustained oxidative stress. Conversely, CAT activity continued to rise across all concentrations, including the highest exposure levels, suggesting its sustained role in mitigating accumulated hydrogen peroxide.

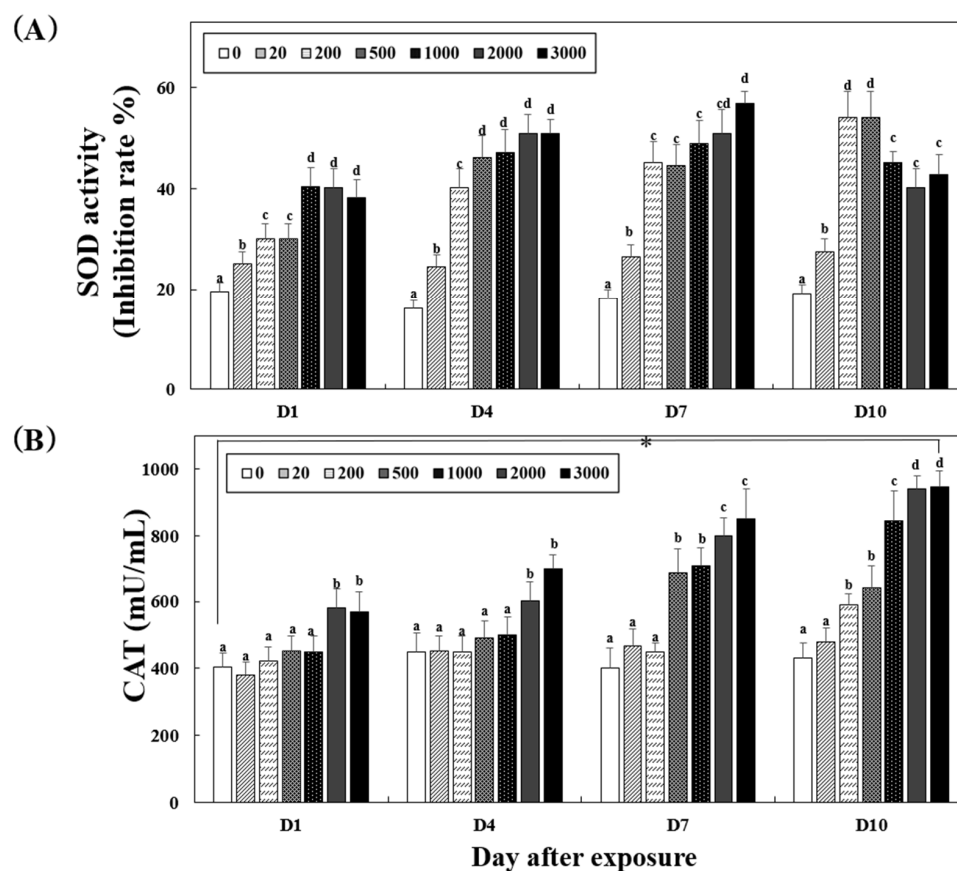


Figure 3. Changes in the superoxide dismutase (SOD) (A) and catalase (CAT) (B) activities in the hepatopancreas of *Haliotis discus hannai* subjected to the following experimental exposure: 0, 20, 200, 500, 1000, 2000, or 3000 mg/L of bentonite clay for 10 d. Different lowercase letters indicate significant differences between different concentrations within the same exposure day ($p < 0.05$). Asterisk (*) indicate the significant interaction between time and concentration ($p < 0.05$). All values are means $\pm$ standard error (SE; $n = 3$).

The two-way ANOVA results supported these observations (Table 2). For SOD, exposure time had a statistically significant effect ($F = 28.397$, $p = 0.012$), while bentonite concentration and the interaction between time and concentration were not significant ($F = 2.064$, $p = 0.102$; interaction $F = 0.954$, $p = 0.688$). For CAT, both time ($F = 30.984$, $p = 0.033$) and concentration ($F = 4.511$, $p = 0.012$) had significant effects, and the interaction term also showed significance ($F = 0.598$, $p = 0.049$), indicating that CAT activity was influenced by the combined effects of time and exposure level.

Table 2. Summary of two-way analysis of variance testing the effects of time (T) and bentonite (B) H_2O_2 , MDA, SOD, CAT, GST, and TAC.

Source	df	H_2O_2		MDA		SOD		CAT		GST		TAC	
		<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Time	3	34.215	0.045	30.144	0.039	28.397	0.012	30.984	0.033	24.196	0.419	31.904	0.187
Bentonite	6	3.495	0.039	1.352	0.044	2.064	0.102	4.511	0.012	3.010	0.042	2.901	0.046
T $\times$ B	18	0.784	0.195	0.894	0.395	0.954	0.688	0.598	0.049	0.606	0.207	0.732	0.721

3.4. Detoxification Enzymatic Activities

GST activity changes with bentonite concentration are shown in Figure 4. GST activity showed a statistically significant increase from day 1 in bentonite treatment groups ($p < 0.05$). A marked elevation in GST activity was observed on day 4 in the 2000 and 3000 mg/L groups. In the 3000 mg/L group, GST activity began to decline after day 4, whereas in the 2000 mg/L group, the highest activity was recorded on day 7, followed by a subsequent decrease in intracellular levels. Both exposure time ($F = 24.196$, $p = 0.419$) and bentonite concentration ($F = 3.010$, $p = 0.042$) significantly affected GST activity (Table 2). However, the interaction between time and concentration was not statistically significant ($F = 0.606$, $p = 0.207$), indicating that the effects of time and concentration were independent in driving GST responses.

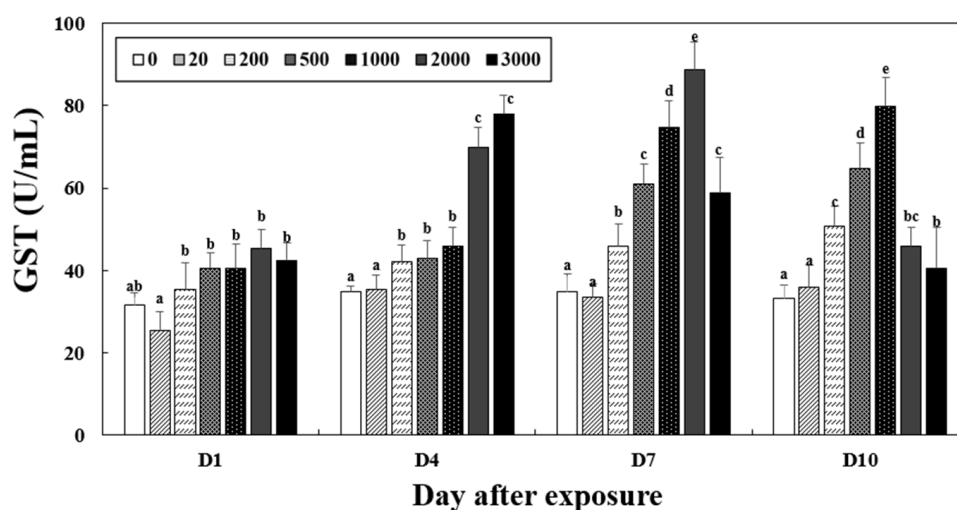


Figure 4. Changes in the glutathione-S-transferase (GST) activity in the hepatopancreas of *Halotis discus hannai* subjected to the following experimental exposure: 0, 20, 200, 500, 1000, 2000, or 3000 mg/L of bentonite clay for 10 d. Different lowercase letters indicate significant differences between different concentrations within the same exposure day ($p < 0.05$). All values are means $\pm$ standard error (SE; $n = 3$).

3.5. Total Antioxidant Capacity

TAC was significantly elevated in all treatment groups compared to the control on day 1 of exposure ($p < 0.05$, Figure 5). However, a marked decrease in TAC was observed in the groups exposed to concentrations of 2000 mg/L or higher from subsequent time points onward. By day 10, the 1000 mg/L group exhibited the highest TAC level (8.96 ± 0.09 mM), while the 2000 mg/L (2.70 ± 0.18 mM) and 3000 mg/L (1.70 ± 0.16 mM) groups showed values that were comparable to or lower than those of the control group.

Both bentonite concentration ($F = 2.901$, $p = 0.046$) and time ($F = 31.904$, $p = 0.187$) were observable, though time was not statistically significant at the $p < 0.05$ level. The interaction between time and concentration was also not significant ($F = 0.732$, $p = 0.721$), suggesting independent effects.

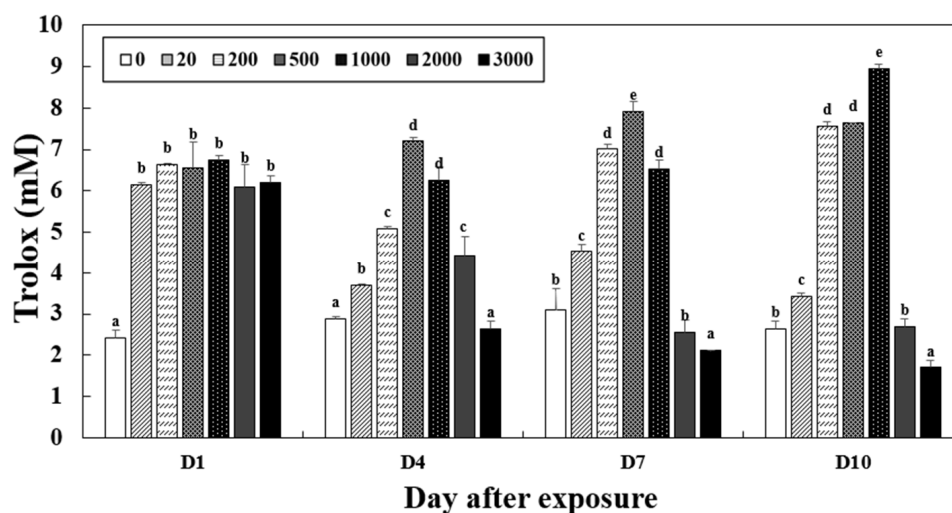


Figure 5. Changes in the Trolox equivalent antioxidant capacity in the hepatopancreas of *Haliotis discus hannai* subjected to the following experimental exposure: 0, 20, 200, 500, 1000, 2000, or 3000 mg/L of bentonite clay for 10 d. Different lowercase letters indicate significant differences between different concentrations within the same exposure day ($p < 0.05$). All values are means $\pm$ standard error (SE; $n = 3$).

4. Discussion

Numerous studies have been conducted to assess the ecological impacts of suspended solids generated by marine development on various marine organisms [2,4,5,7,8,17,21–27]. In the present study, the physiological and ecological effects of bentonite on abalone (*Haliotis discus hannai*), a commercially valuable mollusk species, were investigated.

In the present study, no mortality was observed in abalone exposed to various concentrations of bentonite for up to 7 days. While limited mortality occurred in the high-concentration groups on day 8, no further significant mortality was observed thereafter (Table 1), indicating that bentonite at the tested concentrations may not exert acute lethal effects on abalone in the exposure duration tested during the present study.

Although short-term exposure to environmental stressors does not typically result in immediate mortality in marine organisms, chronic exposure can induce oxidative stress by promoting the excessive generation of reactive oxygen species (ROS) and lipid peroxidation, ultimately leading to organismal death [28]. In response to oxidative stress, organisms activate endogenous antioxidant defense mechanisms, including the induction of antioxidant enzymes such as SOD and CAT, as well as detoxification enzymes like GST. The expression and activity of these enzymes represent an essential cellular defense strategy against environmental fluctuations.

Previous studies have demonstrated that abalone exposed to various environmental stressors—such as chemical pollutants or changes in water temperature—exhibit either upregulation or suppression of antioxidant enzyme activity [9,29]. For instance, Zhang et al. [23] reported that although modified clay did not induce significant acute toxicity in abalone within 96 h of exposure, the expression of SOD and CAT was either elevated or suppressed compared to the control. Wang et al. [24] further hypothesized that with increasing concentrations of suspended sediments, particulates could pass through the mantle during respiration and adhere to the gill surface, thereby impairing respiratory efficiency and compromising physiological function in abalone.

In the present study, bentonite was tested at concentrations up to 3000 mg/L, a level considerably higher than those used in previous suspended particulate experiments [25,26,30]. For instance, Cid et al. [30] examined oxidative responses in the clam *Corbicula fluminea*

exposed to suspended particulate matter at concentrations up to 1000 mg/L. Notably, exposure to bentonite significantly influenced SOD and CAT activity, and elevated levels of H₂O₂ and MDA were observed in treated groups. These results suggest that bentonite exposure under the experimental conditions of the present study induced oxidative stress in abalone, leading to potential cellular damage.

Glutathione S-transferase (GST) is a major detoxification enzyme that plays a key role in protecting organisms from xenobiotic-induced oxidative damage by catalyzing the conjugation of glutathione (GSH) with various toxic compounds [31]. In the present study, GST activity in abalone increased proportionally with higher bentonite concentrations up to day 4, after which a decreasing trend was observed in the high-concentration treatment groups. Similar biphasic patterns in GST activity have been observed in *Crassostrea virginica* exposed to graphene oxide, where GST activity initially increased and subsequently declined over time [32]. Additionally, in *Haliotis discus hannai*, exposure to microplastics and bisphenol A induced significant antioxidant enzyme responses including GST [33]. Further, freshwater snails *Bellamya purificata* exposed to landfill leachate and BPA exhibited approximately 80% increase in GST activity, highlighting the sensitivity of GST as a biomarker to particulate or chemical exposure [34].

These findings support the notion that GST activity in mollusks can exhibit dynamic responses depending on particle type and exposure conditions. This suggests a possible threshold-dependent oxidative stress mechanism. However, the specific response may vary depending on species and pollutant type.

The early induction of GST is presumed to reflect an initial activation of the detoxification system aimed at mitigating oxidative damage by eliminating intracellular ROS. However, sustained oxidative stress may lead to the depletion of its substrate, GSH, ultimately resulting in suppressed GST activity. In this context, the decline in GST activity observed in this study after longer exposure periods may indicate that prolonged exposure to high concentrations of suspended bentonite overwhelmed the antioxidant capacity of abalone, leading to substrate exhaustion and downregulation of detoxification enzymes.

TAC serves as an overall indicator of an organism's antioxidant defense status. In this study, TAC levels were initially elevated across all bentonite-exposed groups, indicating an acute mobilization of non-enzymatic antioxidants (e.g., glutathione, vitamins) in response to oxidative stress. However, a subsequent decline in TAC in high-concentration groups over time suggests depletion of these reserves under chronic exposure conditions.

In contrast, enzymatic antioxidants such as SOD and CAT continued to exhibit elevated activity over time, pointing to sustained enzymatic defense mechanisms. This apparent discrepancy can be explained by their distinct physiological roles: TAC reflects the combined action of both enzymatic and non-enzymatic antioxidants, whereas SOD and CAT are part of the enzymatic system that specifically detoxify superoxide radicals and hydrogen peroxide, respectively [35,36]. Supporting this interpretation, studies on *Mytilus galloprovincialis* exposed to metal stress demonstrated complex patterns of enzymatic versus non-enzymatic antioxidant responses, with initial non-enzymatic activation followed by enzymatic responses over time [37]. Similarly, in snails *Physella acuta* exposed to riverine contaminants, SOD acted as the primary defense, but CAT activity lagged—indicating temporal and functional separation in enzyme-mediated antioxidant pathways [38].

Taken together, these findings underscore that TAC and SOD/CAT are complementary but temporally distinct components of the antioxidant response. While non-enzymatic reserves may be exhausted under prolonged stress—manifesting as declining TAC—enzymatic antioxidants may continue to function until overall defense mechanisms start to fail under persistent oxidative load.

Numerous laboratory and field studies have reported that suspended solids generated from marine development activities generally exhibit low bioavailability and minimal tissue accumulation in marine fauna, particularly benthic organisms [23–25]. However, the specific physiological impacts appear to vary depending on the organism being tested and the physicochemical properties of the suspended particulate material, including the type of clay used. Based on previous findings and the results of the present study, bentonite does not appear to cause acute mortality in abalone under short-term exposure. Nonetheless, the observed oxidative stress response indicates that bentonite may exert sublethal physiological effects, particularly in long-term exposure scenarios.

Therefore, while bentonite may be considered relatively non-toxic in the short term, its potential to induce oxidative stress highlights the need for further long-term ecophysiological studies. While this study provides valuable insights into the oxidative stress response of *H. discus hannai* to suspended bentonite exposure, it is limited in scope to enzymatic antioxidant biomarkers. Further studies incorporating histopathological assessments, gill microbiota profiling, and analysis of mucosal responses are recommended to more fully characterize the sublethal impacts of drilling-derived particulates on this species.

5. Conclusions

This study evaluated the effects of suspended bentonite exposure on the survival and antioxidant defense system of Pacific abalone, *Haliotis discus hannai*. Results showed that bentonite did not induce acute mortality during the first 7 days, and only minor mortality occurred at high concentrations (≥ 500 mg/L) after day 8, suggesting limited short-term lethality. However, bentonite exposure significantly altered oxidative stress biomarkers. Elevated levels of H₂O₂ and MDA indicated increased reactive oxygen species production and lipid peroxidation, while changes in SOD, CAT, and GST activities reflected dynamic antioxidant and detoxification responses. SOD and CAT were initially upregulated, with SOD activity declining under sustained high-concentration exposure, suggesting enzyme inhibition or exhaustion. GST exhibited a biphasic response, with early activation followed by suppression at higher concentrations and longer exposures, likely due to glutathione depletion. Total antioxidant capacity (TAC) initially increased but later declined in high-concentration groups, indicating depletion of non-enzymatic antioxidant reserves.

Overall, these findings suggest that while bentonite does not cause acute lethality in abalone under short-term exposure, it induces sublethal oxidative stress responses that may compromise physiological functions under prolonged or repeated exposure. Given the ecological and economic importance of *H. discus hannai*, these results highlight the need for further long-term ecophysiological and histopathological studies to better understand the potential risks of bentonite and other drilling-derived particulates in marine environments.

Author Contributions: J.A.S.; Investigation, Formal analysis and Writing—original draft, D.M.C.; Methodology and Data curation, Y.-H.J.; Investigation, H.-S.P.; Project administration, T.K.; Resources and Funding acquisition, S.-I.J.; Data curation, D.-W.L.; Resources and Funding acquisition, and Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the Korea Institute of Ocean Science and Technology (PEA0305) and funded by the Ministry of Oceans and Fisheries (RS-2024-00406249), Republic of Korea.

Institutional Review Board Statement: This study did not require Institutional Review Board (IRB) approval. This research utilized invertebrate species (mollusks), which are exempt from ethical approval requirements under South Korea's Animal Protection Act (Law No. 19880).

Data Availability Statement: Dataset available on request from the authors.

Acknowledgments: The authors would like to thank the anonymous reviewers who critically reviewed the manuscript and colleagues who helped with sample collection.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

References

1. Neff, J.M.; Bothner, M.H.; Maciolek, N.J.; Grassle, J.F. Impacts of exploratory drilling for oil and gas on the benthic environment of Georges Bank. *Mar. Environ. Res.* **1989**, *27*, 77–114. [\[CrossRef\]](#)
2. Cranford, P.J.; Gordon, D.C., Jr.; Lee, K.; Armsworthy, S.L.; Tremblay, G.H. Chronic toxicity and physical disturbance effects of water- and oil-based drilling fluids and some major constituents on adult sea scallops (*Placopecten magellanicus*). *Mar. Environ. Res.* **1999**, *48*, 225–256. [\[CrossRef\]](#)
3. Elkatatny, S.; Kamal, M.S.; Alakbari, F.; Mahmoud, M. Optimizing the rheological properties of water-based drilling fluid using clays and nanoparticles for drilling horizontal and multi-lateral wells. *Appl. Rheol.* **2018**, *28*, 201843606. [\[CrossRef\]](#)
4. Strachan, M.F.; Kingston, P.F. A comparative study on the effects of barite, ilmenite and bentonite on four suspension feeding bivalves. *Mar. Pollut. Bull.* **2012**, *64*, 2029–2038. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Cranford, P.J.; Gordon, D.C., Jr. The influence of dilute clay suspensions on sea scallop (*Placopecten magellanicus*) feeding activity and tissue growth. *Nethe. J. Sea Res.* **1992**, *30*, 107–120. [\[CrossRef\]](#)
6. Lee, Y.C.; Jin, E.S.; Jung, S.W.; Kim, Y.M.; Chang, K.S.; Yang, J.W.; Kim, S.W.; Kim, Y.O.; Shin, H.J. Utilizing the algicidal activity of aminoclay as a practical treatment for toxic red tides. *Sci. Rep.* **2013**, *3*, 1292. [\[CrossRef\]](#)
7. Zhang, Y.; Song, X.; Yu, Z.M.; Zhang, P.P.; Cao, X.H.; Yuan, Y.Q. Impact assessment of modified clay on embryo-larval stages of turbot *Scophthalmus maximus* L. *J. Oceanol. Limnol.* **2019**, *37*, 1051–1061. [\[CrossRef\]](#)
8. Archambault, M.C.; Bricelj, V.M.; Grant, J.; Anderson, D.M. Effects of suspended and sedimented clays on juvenile hard clams, *Mercenaria mercenaria*, within the context of harmful algal bloom mitigation. *Mar. Biol.* **2004**, *144*, 553–565. [\[CrossRef\]](#)
9. Wu, C.; Zhang, W.; Mai, K.; Xu, W.; Zhong, X. Effects of dietary zinc on gene expression of antioxidant enzymes and heat shock proteins in hepatopancreas of abalone *Haliotis discus hannai*. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **2011**, *154*, 1–6. [\[CrossRef\]](#)
10. Jiang, Q.; Zhou, Z.; Wang, L.; Shi, X.; Wang, J.; Yue, F.; Yi, Q.; Yang, C.; Song, L. The immunomodulation of inducible nitric oxide in scallop *Chlamys farreri*. *Fish Shellfish Immunol.* **2013**, *34*, 100–108. [\[CrossRef\]](#)
11. Carnevali, S.; Petruzzelli, S.; Longoni, B.; Vanacore, R.; Barale, R.; Cipollini, M.; Scatena, F.; Paggiaro, P.; Celi, A.; Giuntini, C. Cigarette smoke extract induces oxidative stress and apoptosis in human lung fibroblasts. *Am. J. Physiol. Lung Cell Mol. Physiol.* **2003**, *284*, 955–963. [\[CrossRef\]](#)
12. Roch, P. Defense mechanisms and disease prevention in farmed marine invertebrates. *Aquaculture* **1999**, *172*, 125–145. [\[CrossRef\]](#)
13. Park, C.J.; Kim, S.Y. Abalone aquaculture in Korea. *J. Shellfish. Res.* **2013**, *32*, 17–19. [\[CrossRef\]](#)
14. Park, M.; Shin, S.K.; Do, Y.H.; Yarish, C.; Kim, J.K. Application of open water integrated multi-trophic aquaculture to intensive monoculture: A review of the current status and challenges in Korea. *Aquaculture* **2018**, *497*, 174–183. [\[CrossRef\]](#)
15. Song, S.Y.; Park, D.H.; Lee, S.H.; Lim, H.K.; Park, J.W.; Jeong, C.R.; Kim, S.J.; Cho, S.S. Purification of phenoloxidase from *Haliotis discus hannai* and its anti-inflammatory activity in vitro. *Fish Shellfish Immun.* **2023**, *137*, 108741. [\[CrossRef\]](#)
16. Debnath, T.; Mijan, M.A.; Kim, D.H.; Jo, J.E.; Kim, Y.O.; Lee, J.J.; Pyo, H.J.; Lim, B.O. Anti-Inflammatory Effects of *Haliotis discus hannai* I no on Dextran Sulfate Sodium-Induced Colitis in Mice. *J. Food Biochem.* **2015**, *39*, 209–217. [\[CrossRef\]](#)
17. Neff, J.M. Composition, environmental fates, and biological effects of water-based drilling muds and cuttings discharged to the marine environment: A synthesis and annotated bibliography. In Proceedings of the Offshore Operators Committee, Singapore, 20–21 September 2005.
18. Li, Z.; Jiang, M. A Numerical Study for the Drilling Fluid’s Discharge in South China Sea. In Proceedings of the ISOPE International Ocean and Polar Engineering Conference ISOPE, Honolulu, HI, USA, 16–21 June 2019; p. ISOPE-I-19-498.
19. Lee, S.-H.; Jang, S.-C.; Yoon, H.-S. Spatial Distribution Characteristics of Transparency and Suspended Solids in Busan Coastal Waters. *J. Korean Soc. Mar. Environ. Energy* **2022**, *25*, 171–182. [\[CrossRef\]](#)
20. APHA. *Standard Methods for the Examination of Water and Wastewater*, 21st ed.; American Public Health Association: Washington, DC, USA, 2005.
21. Yoon, S.J.; Park, G.S. Ecotoxicological effects of the increased suspended solids on marine benthic organisms. *J. Environ. Sci. Int.* **2011**, *20*, 1383–1394. [\[CrossRef\]](#)
22. Gao, Y.H.; Yu, Z.M.; Song, X.X.; Cao, X.H. Impact of modified clays on the infant oyster (*Crassostrea gigas*). *Mar. Sci. Bull.* **2007**, *26*, 53–60, (In Chinese with English Abstract).
23. Zhang, Y.; Song, X.; Shen, H.; Cao, X.; Yuan, Y.; Wu, Z.; Yu, Z. The effects of modified clay on abalone (*Haliotis discus hannai*) based on laboratory and field experiments. *Environ. Toxicol. Chem.* **2020**, *39*, 2065–2075. [\[CrossRef\]](#)
24. Wang, G.J.; Xie, J.; Yu, D.G.; Wu, L.; Hu, Z.Y. Physiological responses of abalone *Haliotis diversicolor* to suspended sediment stress. *J. Dalian. Fish Univ.* **2007**, *22*, 352–356, (In Chinese with English Abstract).

25. Newcombe, C.P.; MacDonald, D.D. Effects of suspended sediments on aquatic ecosystems. *N. Am. J. Fish. Manag.* **1991**, *11*, 72–82. [[CrossRef](#)]
26. Wilber, D.H.; Clarke, D.G. Biological effects of suspended sediments: A review of suspended sediment impacts on fish and shellfish with relation to dredging activities in estuaries. *N. Am. J. Fish. Manag.* **2001**, *21*, 855–875. [[CrossRef](#)]
27. Hess, S.; Prescott, L.J.; Hoey, A.S.; McMahon, S.A.; Wenger, A.S.; Rummer, J.L. Species-specific impacts of suspended sediments on gill structure and function in coral reef fishes. *Proc. R. Soc. B Biol. Sci.* **2017**, *284*, 20171279. [[CrossRef](#)] [[PubMed](#)]
28. Lesser, M.P. Oxidative stress in marine environments: Biochemistry and physiological ecology. *Annu. Rev. Physiol.* **2006**, *68*, 253–278. [[CrossRef](#)] [[PubMed](#)]
29. Kim, M.J.; Kim, J.A.; Lee, D.W.; Park, Y.S.; Kim, J.H.; Choi, C.Y. Oxidative stress and apoptosis in disk abalone (*Haliotis discus hannai*) caused by water temperature and pH changes. *Antioxidants* **2023**, *12*, 1003. [[CrossRef](#)]
30. Cid, A.; Picado, A.; Correia, J.B.; Chaves, R.; Silva, H.; Caldeira, J.; Diniz, M.S. Oxidative stress and histological changes following exposure to diamond nanoparticles in the freshwater Asian clam *Corbicula fluminea* (Müller, 1774). *J. Hazard. Mater.* **2015**, *284*, 27–34. [[CrossRef](#)]
31. Ni, H.; Peng, L.; Gao, X.; Ji, H.; Ma, J.; Li, Y.; Jiang, S. Effects of maduramicin on adult zebrafish (*Danio rerio*): Acute toxicity, tissue damage and oxidative stress. *Ecotoxicol. Environ. Saf.* **2019**, *168*, 249–259. [[CrossRef](#)]
32. Khan, B.; Adeleye, A.S.; Burgess, R.M.; Russo, S.M.; Ho, K.T. Effects of graphene oxide nanomaterial exposures on the marine bivalve, *Crassostrea virginica*. *Aquat. Toxicol.* **2019**, *216*, 105297. [[CrossRef](#)]
33. Kim, J.A.; Kim, M.J.; Choi, J.Y.; Park, Y.S.; Kim, J.H.; Choi, C.Y. Exposure to bisphenol A and fiber-type microplastics induce oxidative stress and cell damage in disk abalone *Haliotis discus hannai*: Bioaccumulation and toxicity. *Fish Shellfish Immunol.* **2024**, *144*, 109277. [[CrossRef](#)]
34. Li, X.; Lin, L.; Luan, T.; Yang, L.; Lan, C. Effects of landfill leachate effluent and bisphenol A on glutathione and glutathione-related enzymes in the gills and digestive glands of the freshwater snail *Bellamya purificata*. *Chemosphere* **2008**, *70*, 1903–1909. [[CrossRef](#)]
35. Apak, R.; Ozyurek, M.; Guclu, K.; Çapanoğlu, E. Antioxidant activity/capacity measurement. 1. Classification, physicochemical principles, mechanisms, and electron transfer (ET)-based assays. *J. Agric. Food Chem.* **2016**, *64*, 997–1027. [[CrossRef](#)]
36. Haida, Z.; Hakiman, M. A comprehensive review on the determination of enzymatic assay and nonenzymatic antioxidant activities. *Food Sci. Nutr.* **2019**, *7*, 1555–1563. [[CrossRef](#)]
37. Santovito, G.; Trentin, E.; Gobbi, I.; Bisaccia, P.; Tallandini, L.; Irato, P. Non-enzymatic antioxidant responses of *Mytilus galloprovincialis*: Insights into the physiological role against metal-induced oxidative stress. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2021**, *240*, 108909. [[CrossRef](#)]
38. Sedeño-Díaz, J.E.; López-López, E. Oxidative stress in *Physella acuta*: An integrative response of exposure to water from two rivers of Atlantic Mexican slope. *Front. Physiol.* **2022**, *13*, 932537. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.