Antioxidant Response and Oxidative Stress in the Respiratory Tree of Sea Cucumber (*Apostichopus japonicus*) Following Exposure to Crude Oil and Chemical Dispersant

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Abstract: Sea cucumber (*Apostichopus japonicus*) is mainly cultured in the coastal zone, where it is easily threatened by accidental oil spills. Chemical dispersant is one of the efficient oil spill responses for mitigating the overall environmental damage of oil spills. However, the impact of crude oil and chemical dispersants on sea cucumber is less well known. Hence, the present study focused on exploring the antioxidant response and oxidative stress in the respiratory tree of sea cucumber following exposure to GM-2 chemical dispersant (DISP), water-accommodated fractions (WAF), and chemically enhanced WAF (CEWAF) of Oman crude oil for 24 h. Results manifested that WAF exposure caused a significant increase in the reactive oxygen species (ROS) level (5.29 ± 0.30 AU·mgprot⁻¹), and the effect was much more obvious in CEWAF treatment (5.73 ± 0.16 AU·mgprot⁻¹). Total antioxidant capacity (T-AOC), as an important biomarker of the antioxidant defense capacity, showed an increasing trend following WAF exposure (0.95 ± 0.12 U·mgprot⁻¹) while a significant reduction in T-AOC was observed following CEWAF exposure (0.23 ± 0.13 U·mgprot⁻¹). Moreover, we also evaluated the oxidative damage of the macromolecules (DNA, protein, and lipid), and our results revealed that the presence of chemical dispersant enhanced oxidative damage caused by crude oil to sea cucumber.

Keywords: crude oil; chemical dispersant; sea cucumber; acute toxicity; oxidative stress

1. Introduction

The growing global production and consumption of crude oil and its refined products have generated public concern about the effects of potential oil spills on marine ecosystems. Oil slicks from an accidental oil spill can reach coastal habitats through the action of wind, waves, and currents [1–3], and subsequently cause disastrous impacts on coastal zone and human activities. For instance, the 2010 Xingang Port oil spill in Dalian (China) caused more than 1500 t crude oil spilled into the Yellow Sea, resulting in severe losses of marine aquaculture in Liaoning Province. The 2011 Bohai bay oil spill, one of the worst oil pollution incidents along the Chinese coastline, led to more than 840 km² of polluted area and direct economic losses up to CNY12.56 billion in Hebei, Shandong, and Liaoning Province [4]. Chemical dispersants can break up oil slicks into tiny droplets (≤70 µm) in the water column, and then enhance the natural processes of oil slicks, resulting in reducing the amount of oil slicks that might reach coastal habitats [5,6]. Therefore, chemical dispersants have been regarded as one of the efficient
oil spill responses (OSR) for mitigating the overall environmental damage of oil slicks. However, recent studies have shown that the presence of chemical dispersants could also enhance the bioavailability of oil-derived hydrocarbons and then increase potential risk to marine organisms [7–10]. Hence, there are still some dissent from the application of chemical dispersants as an OSR.

Sea cucumber (*Apostichopus japonicas*, Selenka), a typical echinoderm species, is widely distributed along the Northern Pacific coast, e.g., Russia, Japan, and the northern coast of China [11]. Due to its high nutritional and pharmaceutical value, sea cucumber culture has become one of the important aquaculture industries in China, especially in Shandong, Liaoning, and Hebei Province, with total annual production over 210,000 t [12,13]. Currently, sea cucumber is mainly cultured by pond farming, pen culture, and sea ranching in the coastal zone [14]. However, these culture areas are often easily threatened by environmental contaminants derived from human activities, especially from harbors, shipping, offshore exploitation, and other accidental oil spills. Moreover, as an important marine benthos in nutrient recycling, sea cucumber plays an essential role in marine ecosystems [15,16]. Sea cucumber with limited or null motility is generally under greater threat from oil pollution than the mobile species with the ability to escape, such as fishes. Thus, study of the toxic assessments of oil pollution on sea cucumber is essential for marine ecosystems and also have highly applicative significance on OSR planning and decision making.

Crude oil is mainly composed of thousands of organic compounds, of which 75% are hydrocarbons, e.g., polycyclic aromatic hydrocarbons (PAHs) [17]. Previous studies have been documented that oil-derived hydrocarbons could stimulate the generation of reactive oxygen species (ROS) during their biotransformation in marine benthos, leading to an imbalance between the ROS production and the compensatory antioxidant capacity [18,19]. Over-elevation of ROS levels could further induce oxidative stress and cause oxidative damage of the macromolecules (lipids, proteins, and DNA), which has been suggested to be the primary mechanism involved in cell apoptosis and tissue injury of marine benthos following oil-derived hydrocarbons exposure [19–21]. For instance, exposure to water-accommodated fractions (WAF) of Iranian crude oil caused an elevation in ROS level and antioxidant enzyme (glutathione peroxidase [GPx]) activity in Antarctic (*Tigriopus kingsejongensis*) and temperate (*Tigriopus japonicus*) copepods [22]. Our previous work also revealed that exposure to heavy fuel oil caused a significant reduction in total antioxidant capacity (T-AOC) and severe oxidative damage in gonads of adult sea urchin (*Strongylocentrotus intermedius*) [23]. Moreover, recent several studies have reported that the presence of chemical dispersants (chemically enhanced WAF, CEWAF) could enhance the elevation of ROS production and its secondary responses to crude oil in marine benthos, such as marine mussel (*Mytilus galloprovincialis*) [7], eastern oyster (*Crassostrea virginica*) [24], and bay mussel (*Mytilus trossulus*) [25]. However, little is known about the effects of crude oil and chemical dispersants on the antioxidant defense system and oxidative stress in sea cucumber.

Previous research revealed that the respiratory tree, as the primary respiratory organ of sea cucumber, possesses high antioxidant potential and is particularly susceptible to environmental stresses [26,27]. Therefore, in the present study, sea cucumber were acutely exposed to WAF and chemically enhanced WAF (CEWAF) of Oman crude oil, and GM-2 chemical dispersant (DISP) for 24 h. The ROS level, T-AOC, and oxidative damage were measured to assess and compare the antioxidant response and oxidative stress in the respiratory tree of sea cucumber following WAF, CEWAF, and DISP exposure.

2. Materials and Methods

2.1. Preparation of Water-Accommodated Fractions (WAF), Chemically Enhanced WAF (CEWAF), and Chemical Dispersant (DISP) Solutions

Oman crude oil, a light crude oil, was supplied by Dalian Petro Co., Ltd., China. GM-2 chemical conventional dispersant was purchased from Qingdao Guangming Environmental Technology Co., Ltd. The natural seawater (salinity: 32.0 ± 1.0 psu) used for the preparation of WAF, CEWAF, and DISP solutions, sea cucumber maintenance, and acute toxicity tests, was collected locally from the Xinghai
Park, Dalian, China, and pre-filtered through sterile 0.45 µm filters. WAF, CEWAF (Oman crude oil + GM-2), and DISP (GM-2 alone) solutions were prepared according to the Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF) method with some modifications [8,28]. Briefly, WAF solution was prepared with natural seawater at an oil loading rate of 5 g·L⁻¹ in a 10 L glass aspirator bottle (leaving approx. 20% headspace) sealing instantly with a rubber stopper after loading Oman crude oil. The oil-seawater mixture was mixed with a Teflon-coated stirring bar for 18 h and settled for 6 h in the dark at 16.0 ± 0.5 °C to separate the WAF solution from the oil phase. CEWAF solution preparation was performed with natural seawater at an oil loading rate of 5 g·L⁻¹ in a 10 L glass aspirator bottle (leaving approx. 20% headspace). The oil–seawater mixture was mixed with a Teflon-coated stirring bar to form a vortex steadily and then, GM-2 chemical dispersant was sequentially delivered into the center of the vortex at a dispersant-to-oil ratio (DOR) of 1:5 (m/m). The mixture was stirred for 18 h and settled for 6 h in the dark at 16.0 ± 0.5 °C to separate the CEWAF solution from the oil phase. DISP solution preparation followed the same steps as the CEWAF preparation without adding Oman crude oil. All the solutions were freshly prepared prior to the exposure experiments.

2.2. Sea Cucumber Maintenance and Acute Toxicity Tests

Sea cucumber (Apostichopus japonicus, Selenka), with an average wet weight of 56.39 ± 9.38 g, were sourced from Pikou sea cucumber aquaculture zoning, Dalian, China, and acclimatized in an indoor recirculating aquaculture system with a density of 15 per tank (around 60 L capacity). The maintenance conditions were listed as follows: temperature 16.0 ± 0.5 °C, pH 7.9 ± 0.2, salinity 32.0 ± 1.0 psu, dissolved oxygen 7.1 ± 0.3 mg·L⁻¹, and a photoperiod of 14 h light/10 h dark with continuous aeration. Sea cucumber were fed once with formulated feeds per day, and the residual food and feces were siphoned. After a 7-d acclimation period, sea cucumber were randomly allocated to four treatment groups: three exposure groups (DISP, WAF, and CEWAF) and a Control group (pre-filtered natural seawater only) (Supplementary Materials Figure S1). After a 24-h exposure period, the respiratory trees of sea cucumber were dissected and washed with pre-cold 100 mM potassium phosphate (PBS) buffer (pH 7.4). Then, the samples were immediately frozen using liquid nitrogen in the preservation tubes containing 100 mM PBS buffer at a ratio of 1:9 (m/v) for subsequent analysis of biochemical indicators.

2.3. Reactive Oxygen Species (ROS) Production

The ROS production was determined using a 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) method [29] with the Reactive Oxygen Species Assay Kit (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer’s protocol. Briefly, fresh tissues were washed with pre-cold 100 mM PBS buffer (pH 7.4) twice and then homogenized in pre-cold PBS buffer. The homogenate was centrifuged for 20 min at 500× g, and the precipitate was collected. Then, the precipitate was incubated with 100 µL PBS (pH 7.4) and 50 µL DCFH-DA (1 mM) over 60 min at 37 °C. The fluorescence intensity was monitored with excitation at 485 nm and emission at 525 nm using a SpectraMax M5 multimode microplate reader (Molecular Devices, USA). The ROS level was normalized to protein concentration for each sample, which was measured at 595 nm with the Total Protein Assay Kit (Bradford method) (Nanjing Jiancheng Bioengineering Institute, China). The ROS level was expressed in arbitrary units per mg protein (AU·mgprot⁻¹).

2.4. Antioxidant Capacity

The total antioxidant capacity (T-AOC) was measured using the ferric reducing ability of plasma (FRAP) method [30] with the Total Antioxidant Capacity Assay Kit (Nanjing Jiancheng Bioengineering Institute, China). In brief, tissue sample with PBS buffer was homogenized on the ice and centrifuged for 15 min at 3000 rpm, and then the supernate was collected. 10 µL of supernate, 20 µL of peroxidase application solution, and 170 µL of ABTS working solution were added into a 96 well microplate and mixed fully. The mixture was incubated for 6 min at room temperature (24 °C). The optical density
(OD) value was monitored at 520 nm using a microplate ultraviolet–visible (UV-Vis) spectrophotometer (BioTek, USA). The T-AOC was expressed as units per mg protein (U·mgprot⁻¹).

2.5. Oxidative Damage Assessment

Oxidative DNA damage was assessed according to an 8-hydroxy-2′-deoxyguanosine (8-OHdG) method [31] with the 8-OHdG enzyme-linked immunosorbent assay (ELISA) kit (Nanjing Jiancheng Bioengineering Institute, China). According to the manufacturer’s instruction, 50 µL of the tissue sample homogenate was added with 50 µL of the horseradish peroxidase (HRP) conjugated 8-OHdG antibody into a microtiter ELISA plate well, which had been pre-coated with an antibody specific for 8-OHdG. The plate was incubated for 1 h at 37 °C. After incubation, the well was washed five times with 300 µL washing buffer. Then, chromogenic solutions were added and incubated at 37 °C for 15 min. The enzymatic color reaction was terminated by adding 50 µL of 2 M H₂SO₄. The OD value of each well was detected at 450 nm. The 8-OHdG level was determined for each sample from a standard curve and expressed as ng per mg protein (ng·mgprot⁻¹).

Protein oxidation was determined based on the level of protein carbonyls (PCO) according to the 2,4-dinitrophenylhydrazide (DNPH) method [32,33] with the Protein Carbonyl Assay Kit (Nanjing Jiancheng Bioengineering Institute, China). Briefly, 100 µL of the tissue sample homogenate was incubated with 10 mM DNPH (in 2 N HCl) in the dark at 37 °C for 30 min. An equivalent volume of trichloroacetic acid (TCA) was added and centrifuged for 10 min at 12,000 rpm. The precipitate was washed four times with ethanol/ethyl acetate (1:1, v/v) mixture. The final precipitate was redissolved in 6 M guanidine hydrochloride and incubated at 37 °C for 15 min. After a 15-min centrifugation at 12,000 rpm, the OD value of the final supernate was measured at 370 nm. The PCO level was calculated based on the protein concentration for each sample and expressed as nanomoles per mg protein (nmol·mgprot⁻¹).

Lipid peroxidation was evaluated using a malondialdehyde (MDA) method described by Buege et al. [34] with the Malondialdehyde Assay Kit (thiobarbituric acid (TBA) method) following the manufacturer’s protocol (Nanjing Jiancheng Bioengineering Institute, China). Briefly, 500 µL of the tissue sample homogenate and 500 µL of TBA were added into a microcentrifuge tube and mixed fully. The reaction mixture was incubated at 95 °C for 60 min and cooled to room temperature in an ice bath for 10 min. The OD value of the reaction mixture was measured at 532 nm. The MDA concentration was expressed as nanomoles per mg protein (nmol·mgprot⁻¹).

2.6. Integrated Biomarker Response (IBR) Index

To further compare the relative values of oxidative stress responses in DISP, WAF, and CEWAF treatments, integrated biomarker response (IBR) index was applied as described by Beliaeff Benoit et al. [35] and modified by Sanchez Wilfried et al. [36] and Vieira et al. [37]. In the present study, the deviation between biomarkers measured in different treatments (Xᵢ) were compared to those measured in the Control (X₀). For each biomarker, the ratio of Xᵢ and X₀ was log-transformed (Yᵢ). Then, the general mean (µ) and standard deviation (s) were calculated in each group. Yᵢ values were standardized by the formula: Zᵢ = (Yᵢ − µ)/s and the difference between Zᵢ and Z₀ (the Control) was used to define the biomarker deviation index (A). To obtain an IBR index, the A value of each biomarker was calculated for each treatment and the IBR index was determined by the sum of the absolute values of A.

2.7. Chemicals Analysis

Total petroleum hydrocarbons (TPH) concentration was measured by ultraviolet spectrometry using the Standardization Administration of China (SAC) GB 17378.4-2007 method with some modifications as described in our previous work [38,39]. Briefly, 200 mL water sample was extracted with 20 mL n-hexane (with a transmittance over 90% at 225 nm wavelength) in a separatory funnel. The absorbance of n-hexane extract was detected at 225 nm by the UV-Vis spectrophotometer in
a quartz cuvette. The TPH concentration was determined based on the calibration curve (WAF: \( r^2 = 0.999; \) CEWAF: \( r^2 = 0.999; \) Figure 1A). Moreover, 16 priority PAHs listed by US Environmental Protection Agency (EPA) were also analyzed in the present study as described in our previous work [38], including naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Fle), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fla), pyrene (Pyr), benzo[a]anthracene (B[a]A), chrysene (Chr), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo[a]pyrene (B[a]P), indeno[1,2,3-cd]pyrene (I[123-cd]P), dibenzo[a,h]anthracene (D[a]hA), and benzo[g,h,i]perylene (B[ghi]P) [40]. Water sample was pre-processed according to the EPA 3510C liquid-liquid extraction method [42]. Cleanup of the concentrate was performed based on the EPA 3630C silica gel cleanup method [41]. Then, PAHs were analyzed using an Agilent 7890B gas chromatography coupled 5977A mass selective detector according to ISO 28540:2011 [43].

![Figure 1](image-url) Figure 1. (A) Standard curves of total petroleum hydrocarbons (TPH) concentration for water-accommodated fractions (WAF, black filled circle) and chemically enhanced WAF (CEWAF, unfilled triangle up) solutions. Standard curves were fitted using the linear fitting model and are denoted using solid lines. Short dash lines represent the 95% confidence interval. (B) Proportion (%) of 16 priority polycyclic aromatic hydrocarbons (ΣPAHs) listed by the US Environmental Protection Agency (EPA) in WAF and CEWAF solutions.

### 2.8. Statistical Analysis

All data were presented as the mean ± standard deviation (SD). Statistical difference between the treatments and the Control was analyzed via the one-way analysis of variance (one-way ANOVA). Significant differences were accepted when \( p < 0.05 \). The asterisks (*, **, or ***) in the graphics denoted statistically significant difference from the Controls (\( p < 0.05, 0.01, \) or 0.001, respectively). Statistical analysis and graph plotting were performed using SigmaPlot Ver 14.0 (Systat Software, Inc., USA).

### 3. Results

#### 3.1. Analytical Chemistry

The concentrations of TPH were 4.25 ± 0.07 and 57.81 ± 1.15 mg·L\(^{-1}\) in WAF and CEWAF solutions, respectively, indicating that the TPH level in CEWAF solution was 13.60 fold higher than that in WAF solution. The concentrations of total 16 priority PAHs (ΣPAHs) in WAF and CEWAF solutions were 2.25 ± 0.43 and 5.68 ± 0.82 μg·L\(^{-1}\), respectively. The ΣPAHs concentration in CEWAF solution was 2.52 fold higher than that in WAF solution. Moreover, the proportions (%) of each PAH showed various changes between WAF and CEWAF solutions. In WAF solution, the most abundant components were lower molecular weight (LMW) PAHs (2-ringed) accounting for 57.9% of ΣPAHs, followed by higher molecular weight (HMW) PAHs, including 3-ringed (15.5%), 4-ringed (13.6%) and ≥5-ringed (13.0%) PAHs. In CEWAF solution, the LMW PAHs (46.3%) were still the most abundant components but showed a relatively lower proportion than that in WAF solution, accompanied by a corresponding
increase in the proportion of HMW PAHs, including 3-ringed PAHs accounting for 18.6%, 4-ringed PAHs accounting for 17.7%, and ≥5-ringed PAHs accounting for 17.4% (Figure 1B).

3.2. Survival Rates

During the 24-h acute exposure period, WAF, CEWAF or DISP treatments did not cause a reduction in the survival rates of sea cucumber compared to the Control (Chi-square, \( p > 0.05 \), Supplementary Materials Figure S2).

3.3. ROS Levels

No statistical difference was observed between the DISP treatment (4.53 ± 0.75 AU·mgprot\(^{-1}\)) and the Control (4.65 ± 0.21 AU·mgprot\(^{-1}\)) (one-way ANOVA, \( p = 0.94 > 0.05 \)), manifesting that acute exposure to DISP had no obvious impact on the ROS level in sea cucumber (Figure 2). The ROS levels in WAF and CEWAF treatments were 5.29 ± 0.30 and 5.73 ± 0.16 AU·mgprot\(^{-1}\), respectively, indicating that both WAF and CEWAF exposure caused a significant increase in ROS levels compared with the Control (one-way ANOVA, \( p = 0.03 < 0.05 \) and \( p < 0.001 \) for WAF and CEWAF, respectively). Moreover, the ROS level in CEWAF treatment was also much higher than that in the WAF (one-way ANOVA, \( p = 0.002 < 0.01 \)).

![Figure 2](image)

Figure 2. Reactive oxygen species (ROS) levels in the respiratory tree of sea cucumber following GM-2 chemical conventional dispersant (DISP, gray filled), water-accommodated fractions (WAF, dark-gray filled), and chemically enhanced WAF (CEWAF, light-gray filled) of Oman crude oil. The Control was the pre-filtered natural seawater only (black filled). Asterisks (*, or ***) denote significant differences between the treatments and the Control (\( p < 0.05 \) or 0.001, respectively). Dark traits denote significant differences among the treatments (one-way analysis of variance (ANOVA)).

3.4. Antioxidant Capacity

To assess the antioxidant defense capacity in the respiratory tree of sea cucumber following the DISP, WAF, and CEWAF exposure, T-AOC was measured in the present study (Figure 3). Results showed that compared with the Control (0.82 ± 0.14 U·mgprot\(^{-1}\)), DISP had no significant impact on the T-AOC (0.79 ± 0.13 U·mgprot\(^{-1}\); one-way ANOVA, \( p = 0.99 > 0.05 \)). WAF exposure caused a slight increase in the T-AOC (0.95 ± 0.12 U·mgprot\(^{-1}\)) but without significant difference compared with the Control (one-way ANOVA, \( p = 0.65 > 0.05 \)); while CEWAF exposure induced an extremely significant decrease in the T-AOC (0.23 ± 0.13 U·mgprot\(^{-1}\)) (one-way ANOVA, \( p < 0.001 \)). Meanwhile, the T-AOC in CEWAF treatment was also much lower than that in WAF treatment (one-way ANOVA, \( p < 0.001 \)).
WAF and CEWAF treatments were much higher than that in the Control (one-way ANOVA, while the MDA content in DISP treatment was subtly lower than that in the Control but without (one-way ANOVA, p < 0.05). Moreover, the MDA content in CEWAF treatment was also much higher than that in WAF treatment (one-way ANOVA, p < 0.001).

3.5. Oxidative Damage

To further evaluate the oxidative damage of the macromolecules (DNA, proteins, and lipids) after DISP, WAF, and CEWAF exposure, the levels of 8-OHdG, PCO, and MDA in the respiratory tree of sea cucumber were detected in the present study. For oxidative DNA damage, the standard curve of 8-OHdG level was fitted using the nonlinear logistic model with four parameters (r² = 0.972; Figure 4A). Results showed that no significant differences were found for 8-OHdG levels for the three treatments compared with the Control (one-way ANOVA, p = 0.68, 0.61, and 0.18 > 0.05 for DISP, WAF, and CEWAF, respectively; Figure 4B), indicating that none of DISP, WAF, or CEWAF exposure caused significant change in the levels of oxidative DNA damage. Regarding the protein oxidation, the levels of PCO were determined (Figure 4C). Results manifested that both DISP and WAF exposure induced a slight increase in the PCO levels but without significant differences compared with the Control (one-way ANOVA, p = 0.92 and 0.19 > 0.05 for DISP and WAF, respectively); while CEWAF exposure produced a significant elevation of the PCO level (one-way ANOVA, p = 0.04 < 0.05). As for lipid peroxidation, MDA contents were measured (Figure 4D). Results revealed that the MDA contents in WAF and CEWAF treatments were much higher than that in the Control (one-way ANOVA, p < 0.01); while the MDA content in DISP treatment was subtly lower than that in the Control but without significant difference (one-way ANOVA, p = 0.67 > 0.05). Moreover, the MDA content in CEWAF treatment was also much higher than that in WAF treatment (one-way ANOVA, p < 0.001).

Figure 3. Total antioxidant capacity (T-AOC) in the respiratory tree of sea cucumber following GM-2 chemical conventional dispersant (DISP, gray filled), water-accommodated fractions (WAF, dark-gray filled) and chemically enhanced WAF (CEWAF, light-gray filled) of Oman crude oil. The Control was the pre-filtered natural seawater only (black filled). Asterisks (***') denote significant differences between the treatments and the Control (p < 0.001). Dark traits denote significant differences among the treatments (one-way ANOVA).

Figure 4. Cont.
Figure 4. (A) Standard curve of 8-OHdG level (solid line) was fitted using the non-linear logistic model with four parameters. Short dash lines represent the 95% confidence interval. Black-filled diamonds represent the optical density (OD) values. The levels of 8-OHdG (B), protein carbonyls (PCO) (C), and malondialdehyde (MDA) (D) in the respiratory tree of sea cucumber following GM-2 chemical conventional dispersant alone (DISP, gray filled), water-accommodated fractions (WAF, dark-gray filled) and chemically enhanced WAF (CEWAF, light-gray filled) of Oman crude oil. The Control was the pre-filtered natural seawater only (black filled). Asterisks denote significant differences between the treatments and the Control (*, **, or *** denote significant differences among the treatments (one-way ANOVA).

3.6. IBR Index

IBR indexes indicated that CEWAF exposure caused greater variations in biomarker responses, with an average IBR index of 7.71, followed by WAF exposure with an average IBR index of 1.92 (Figure 5). DISP exposure induced a slight variation in biomarker responses, with an average IBR index of 0.76, which was attributed to the changes in MDA content. IBR index results revealed that CEWAF exposure promoted more pronounced effects in biomarker responses related to the oxidative stress than DISP and WAF did.
The use of chemical dispersants following an accidental oil spill could enhance the natural process (e.g., dissolution and microbial biodegradation) of petroleum hydrocarbons by reducing the interfacial tension between oil slicks and water, and mitigate the impact of oil slicks on the coastal habitats and human activities [44]. Nevertheless, chemical dispersion could also lead to the dissolution of more oil-derived hydrocarbons from oil droplets into the water column and then raise the threat of dispersed oil components to marine organisms, especially to marine benthos [24,45]. Sea cucumber, as a common member of marine benthic communities and an important marine aquaculture species, is mainly cultured in the coastal zone, where it is easily threatened by oil pollution. However, the impact of crude oil and chemical dispersant on sea cucumber is still less known. Hence, we exposed sea cucumber to DISP, WAF, and CEWAF solutions for 24 h, and measured the biochemical parameters related to oxidative stress responses to evaluate and compare the toxic effects of crude oil and chemically dispersed crude oil on sea cucumber.

Results manifested that ROS production increased in the respiratory tree of sea cucumber exposed to WAF, and the effect was much more obvious when exposed to CEWAF. The increase in ROS production observed in the present study is consistent with recent reports supporting that oil-derived hydrocarbons could elevate ROS production and the addition of chemical dispersant could enhance the elevation of ROS production in marine benthos, such as marine mussel (*Mytilus galloprovincialis*) [7] and eastern oyster (*Crassostrea virginica*) [24]. It is well known that the antioxidant enzymatic system is the primary defense line to scavenge the excessive ROS and maintain the cellular redox homeostasis within certain limits [46,47]. Consistently, T-AOC, as an important biomarker for assessing the antioxidant defense capacity, showed an increasing trend following WAF exposure in the present study. Differently, a significant reduction in T-AOC was observed following CEWAF exposure compared with the Control, indicating that CEWAF exposure could produce severe damage to the antioxidant defense system in the respiratory tree of sea cucumber. Previous studies have reported that exposure to oil-derived hydrocarbons at relative high concentrations could compromise the ability of antioxidant defense system in marine benthos, which exhibited a significant reduction in the antioxidant enzymatic activities, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [19,23,48], or in the T-AOC used in the present study, which is an integrated parameter to evaluate the cumulative action of all the antioxidants present in vivo [49]. The damage of the antioxidant defense system

**Figure 5.** Integrated biomarker response (IBR) indexes for the respiratory tree of sea cucumber following GM-2 chemical conventional dispersant alone (DISP, A), water-accommodated fractions (WAF, B) and chemically enhanced WAF (CEWAF, C) of Oman crude oil. Biomarker results are denoted in relation to the Control (the pre-filtered natural seawater only) (dash lines). The area above 0 represents the induction of the biomarker, and below 0 denotes the reduction of the biomarker. ROS: reactive oxygen species; T-AOC: total antioxidant capacity; 8-OHdG: 8-hydroxy-2′-deoxyguanosine; PCO: protein carbonyls; MDA: malondialdehyde.

### 4. Discussion

The use of chemical dispersants following an accidental oil spill could enhance the natural process (e.g., dissolution and microbial biodegradation) of petroleum hydrocarbons by reducing the interfacial tension between oil slicks and water, and mitigate the impact of oil slicks on the coastal habitats and human activities [44]. Nevertheless, chemical dispersion could also lead to the dissolution of more oil-derived hydrocarbons from oil droplets into the water column and then raise the threat of dispersed oil components to marine organisms, especially to marine benthos [24,45]. Sea cucumber, as a common member of marine benthic communities and an important marine aquaculture species, is mainly cultured in the coastal zone, where it is easily threatened by oil pollution. However, the impact of crude oil and chemical dispersant on sea cucumber is still less known. Hence, we exposed sea cucumber to DISP, WAF, and CEWAF solutions for 24 h, and measured the biochemical parameters related to oxidative stress responses to evaluate and compare the toxic effects of crude oil and chemically dispersed crude oil on sea cucumber.

Results manifested that ROS production increased in the respiratory tree of sea cucumber exposed to WAF, and the effect was much more obvious when exposed to CEWAF. The increase in ROS production observed in the present study is consistent with recent reports supporting that oil-derived hydrocarbons could elevate ROS production and the addition of chemical dispersant could enhance the elevation of ROS production in marine benthos, such as marine mussel (*Mytilus galloprovincialis*) [7] and eastern oyster (*Crassostrea virginica*) [24]. It is well known that the antioxidant enzymatic system is the primary defense line to scavenge the excessive ROS and maintain the cellular redox homeostasis within certain limits [46,47]. Consistently, T-AOC, as an important biomarker for assessing the antioxidant defense capacity, showed an increasing trend following WAF exposure in the present study. Differently, a significant reduction in T-AOC was observed following CEWAF exposure compared with the Control, indicating that CEWAF exposure could produce severe damage to the antioxidant defense system in the respiratory tree of sea cucumber. Previous studies have reported that exposure to oil-derived hydrocarbons at relative high concentrations could compromise the ability of antioxidant defense system in marine benthos, which exhibited a significant reduction in the antioxidant enzymatic activities, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [19,23,48], or in the T-AOC used in the present study, which is an integrated parameter to evaluate the cumulative action of all the antioxidants present in vivo [49]. The damage of the antioxidant defense system
could lead to insufficient capacity for scavenging ROS and then a significant net increase in ROS levels, which subsequently could result in extensive oxidative damage of the macromolecules (DNA, proteins, and lipids) [50,51]. In the present study, we also evaluated the oxidative damage of these macromolecules in the respiratory tree of sea cucumber based on the levels of 8-OHdG, PCO, and MDA. We found that CEWAF exposure could produce a more significant increase in the PCO and MDA levels than did WAF exposure, which was accounted for 1.13- and 1.93-fold change, respectively. DISP exposure caused no apparent impact on the PCO and MDA levels. Besides, no increase in the level of oxidative DNA damage (8-OHdG) was observed in DISP, WAF, and CEWAF treatments. Our data suggested that chemically dispersed crude oil could enhance the oxidative damage in the respiratory tree of sea cucumber, which mainly manifested as protein oxidation and lipid peroxidation. Furthermore, the change in MDA level was much larger than the PCO level caused by WAF and CEWAF exposure, implying that lipid peroxidation could be the relatively more susceptible bioindicator of ROS-generated oxidative damage caused by oil-derived hydrocarbons exposure.

Several studies recently published on comparative analysis of toxic effects between WAF and CEWAF on various marine organisms have reported that chemical dispersant could increase the bioavailability of dissolved oil hydrocarbons and then enhance the toxicity of crude oil on marine organisms [9,10,52]. Our chemical analysis results manifested that both TPH content (around 13.60-fold) and PAHs level (around 2.52-fold) in CEWAF were higher than those in WAF. Moreover, compared with WAF, the proportion of HMW PAHs is much higher in CEWAF, especially in 3-ringed PAHs, which has been reported to be the predominant toxic components of oil-derived hydrocarbons to marine organisms [53,54]. It has also been documented that PAHs could elevate the ROS generation during their metabolism and biotransformation processes in marine benthos, leading to an imbalance between the ROS generation and scavenging [18,19,55,56]. Likewise, the ΣPAHs concentrations in WAF and CEWAF coincided with the oxidative damage observed in the present study. Consequently, our results supported these previous findings that the enhancement of the oxidative damage in marine benthos caused by oil-derived hydrocarbons could be attributed to the presence of chemical dispersants that solubilize more hydrocarbons from oil droplets into the water column, resulting in elevating their bioavailability to marine benthos.

Overall, the present study focused on comparative analysis of the antioxidant response and oxidative stress in the respiratory tree of sea cucumber following acute exposure to DISP, WAF, and CEWAF. Our results revealed that chemical dispersant enhanced oxidative damage caused by crude oil to sea cucumber, which could be ascribed to its ability to solubilize higher levels of oil-derived hydrocarbons into the water column. Our data also suggested that the application of chemical dispersant in oil spill could increase the exposure of marine benthos to crude oil. Consistently, previous studies have reported that the application of chemical dispersant could also enhance the toxic effects of spilled oil to other marine organisms, such as plankton communities [57] and fishes [58]. Hence, comprehensive consideration of ecological effects of the application of chemical dispersant as an OSR is essential in OSR planning and decision making during accidental oil spills. Also, the potential health risk of oil and chemical dispersant to the respiratory system of humans, especially oil-cleaning rescue workers, should be considered before using the chemical dispersant [59]. Our findings on the oxidative responses associated with acute exposure to crude oil and chemically dispersed oil could provide a theoretical basis and guidance for the health management of sea cucumber culture. Future research should work on the investigation of the underlying mechanisms involved in oxidative stress response of sea cucumber to oil-derived hydrocarbons.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2077-1312/8/8/547/s1, Figure S1: Experimental design of sea cucumber following DISP, WAF, and CEWAF exposure, Figure S2: Survival rates of sea cucumber exposed to DISP, WAF, and CEWAF solutions.

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**References**

1. Gao, Y.; Xiong, D.; Qi, Z.; Li, X.; Ju, Z.; Zhuang, X. Distribution of polycyclic aromatic hydrocarbons in sunken oils in the presence of chemical dispersant and sediment. *J. Mar. Sci. Eng.* 2019, 7, 282. [CrossRef]

2. Gao, Y.; Zhao, X.; Ju, Z.; Yu, Y.; Qi, Z.; Xiong, D. Effects of the suspended sediment concentration and oil type on the formation of sunken and suspended oils in the Bohai Sea. *Environ. Sci.-Process Impacts* 2018, 20, 1404–1413. [CrossRef] [PubMed]

3. Yu, Y.; Qi, Z.; Fu, S.; Yu, X.; Li, W.; Xiong, D. Effects of wave conditions and particle size on the release of oil from oil-contaminated sediments in a wave tank. *J. Mar. Sci. Eng.* 2019, 7, 256. [CrossRef]

4. Pan, G.; Qiu, S.; Liu, X.; Hu, X. Estimating the economic damages from the Penglai 19-3 oil spill to the Yantai fisheries in the Bohai Sea of northeast China. *Mar. Pol.* 2015, 62, 18–24. [CrossRef]

5. National Research Council of the National Academies. *Oil Spill Dispersants: Efficacy and Effects*; The National Academies Press: Washington, DC, USA, 2005; p. 396.

6. Prince, R.C. Oil spill dispersants: Boon or bane? *Environ. Sci. Technol.* 2015, 49, 6376–6384. [CrossRef]

7. Katsumiti, A.; Nicolussi, G.; Bilbao, D.; Prieto, A.; Etxebarria, N.; Cajaraville, M.P. In vitro toxicity testing in hemocytes of the marine mussel *Mytilus galloprovincialis* (L.) to uncover mechanisms of action of the water accommodated fraction (WAF) of a naphthenic North Sea crude oil without and with dispersant. *Sci. Total Environ.* 2019, 670, 1084–1094. [CrossRef]

8. Li, X.; Ding, G.; Xiong, Y.; Ma, X.; Fan, Y.; Xiong, D. Toxicity of water-accommodated fractions (WAF), chemically enhanced WAF (CEWAF) of Oman crude oil and dispersant to early-life stages of zebrafish (*Danio rerio*). *Bull. Environ. Contam. Toxicol.* 2018, 101, 314–319. [CrossRef]

9. Mu, J.; Jin, F.; Ma, X.; Lin, Z.; Wang, J. Comparative effects of biological and chemical dispersants on the bioavailability and toxicity of crude oil to early life stages of marine medaka (*Oryzias melastigma*). *Environ. Toxicol. Chem.* 2014, 33, 2576–2583. [CrossRef]

10. Lee, K.-W.; Shim, W.J.; Yim, U.H.; Kang, J.-H. Acute and chronic toxicity study of the water accommodated fraction (WAF), chemically enhanced WAF (CEWAF) of crude oil and dispersant in the rock pool copepod *Tigriopus japonicus*. *Chemosphere* 2013, 92, 1161–1168. [CrossRef] [PubMed]

11. Hamel, J.-F.; Mercier, A. Population status, fisheries and trade of sea cucumbers in temperate areas of the Northern Hemisphere. In *Sea Cucumbers: A Global Review of Fisheries and Trade*; FAO Fisheries and Aquaculture Technical Paper: Rome, Italy, 2008; pp. 257–292.

12. Fisheries and Fisheries Administration Bureau of the Ministry of Agriculture. *China Fishery Statistical Yearbook 2018*; China Agrculture Press: Beijing, China, 2018; p. 181.

13. Huo, D.; Sun, L.; Zhang, L.; Ru, X.; Liu, S.; Yang, X.; Yang, H. Global-warming-caused changes of temperature and oxygen alter the proteomic profile of sea cucumber *Apostichopus japonicus*. *J. Proteom.* 2019, 193, 27–43. [CrossRef]

14. Xia, S.; Yang, H.; Li, Y.; Liu, S.; Zhou, Y.; Zhang, L. Effects of different seaweed diets on growth, digestibility, and ammonia-nitrogen production of the sea cucumber *Apostichopus japonicus* (Selenka). *Aquaculture* 2012, 338–341, 304–308. [CrossRef]

15. Purcell, S.; Conand, C.; Uthicke, S.; Byrne, M. Ecological roles of exploited sea cucumbers. *Oceanogr. Mar. Biol.* 2016, 54, 367–386.

16. Ding, K.; Zhang, L.; Sun, L.; Lin, C.; Feng, Q.; Zhang, S.; Yang, H.; Brinkman, R.; Lin, G.; Huang, Z. Transcriptome analysis provides insights into the molecular mechanisms responsible for evisceration behavior in the sea cucumber *Apostichopus japonicus*. *Comp. Biochem. Physiol. D-Genom.* 2019, 30, 143–157. [CrossRef] [PubMed]
OSPAR guidelines for monitoring the environmental impact of offshore oil and gas activities. In Proceedings of the Meeting of the OSPAR Offshore Industries Committee (OIC), London, UK, 28–30 September 2004; pp. 1–19.

Solé, M.; Lima, D.; Reis-Henriques, M.A.; Santos, M.M. Stress biomarkers in juvenile senegal sole, *Solea senegalensis*, exposed to the water-accommodated fraction of the “Prestige” fuel oil. *Bull. Environ. Contam. Toxicol.* 2008, 80, 19–23. [CrossRef] [PubMed]

Barbosa, D.B.; Mello, A.d.A.; Allodi, S.; de Barros, C.M. Acute exposure to water-soluble fractions of marine diesel oil: Evaluation of apoptosis and oxidative stress in an ascidian. *Chemosphere* 2018, 211, 308–315. [CrossRef]

Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M.T.D.; Mazur, M.; Telser, J. Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.* 2007, 39, 44–84. [CrossRef]

Hannam, M.L.; Bamber, S.D.; John Moody, A.; Galloway, T.S.; Jones, M.B. Immunotoxicity and oxidative stress in the Arctic scallop *Chlamys islandica*: Effects of acute oil exposure. *Ecotoxocol. Environ. Saf.* 2010, 73, 1440–1448. [CrossRef]

Han, J.; Kim, H.-S.; Kim, I.-C.; Kim, S.; Hwang, U.-K.; Lee, J.-S. Effects of water accommodated fractions (WAFs) of crude oil in two congeneric copepods *Tigriopus* sp. *Ecotoxicol. Environ. Saf.* 2017, 145, 511–517. [CrossRef]

Duan, M.; Xiong, D.; Gao, Y.; Bai, X.; Xiong, Y.; Gao, X.; Ding, G. Transgenerational effects of heavy fuel oil on the sea urchin *Strongylocentrotus intermedius* considering oxidative stress biomarkers. *Mar. Environ. Res.* 2018, 141, 138–147. [CrossRef]

Jasperse, L.; Levin, M.; Tsantiris, K.; Smolowitz, R.; Perkins, C.; Ward, J.E.; De Guise, S. Comparative toxicity of Corexit® 9500, oil, and a Corexit®/oil mixture on the eastern oyster, *Crassostrea virginica* (Gmelin). *Aquat. Toxicol.* 2018, 203, 10–18. [CrossRef]

Counihan, K.L. The physiological effects of oil, dispersant and dispersed oil on the bay mussel, *Mytilus trossulus*, in Arctic/Subarctic conditions. *Aquat. Toxicol.* 2018, 199, 220–231. [CrossRef] [PubMed]

Telahigue, K.; Rabeh, I.; Bejaoui, S.; Hajji, T.; Nechi, S.; Chelbi, E.; El Cafsi, M.h.; Soudani, N. Mercury disrupts redox status, up-regulates metallothionein and induces genotoxicity in respiratory tree of sea cucumber (*Holothuria forskali*). *Drug Chem. Toxicol.* 2020, 43, 287–297. [CrossRef] [PubMed]

Huo, D.; Sun, L.; Zhang, L.; Ru, X.; Liu, S.; Yang, H. Metabolome responses of the sea cucumber *Apostichopus japonicus* to multiple environmental stresses: Heat and hypoxia. *Mar. Pollut. Bull.* 2019, 138, 407–420. [CrossRef] [PubMed]

Singer, M.M.; Aurand, D.; Bragin, G.E.; Clark, J.R.; Coelho, G.M.; Sowby, M.L.; Tjeerdema, R.S. Standardization of the preparation and quantitation of water-accommodated fractions of petroleum for toxicity testing. *Mar. Pollut. Bull.* 2000, 40, 1007–1016. [CrossRef]

LeBel, C.P.; Ischiropoulos, H.; Bondy, S.C. Evaluation of the probe 2’,7’-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. *Chem. Res. Toxicol.* 1992, 5, 227–231. [CrossRef]

Benzie, I.F.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Anat. Biochem.* 1996, 239, 70–76. [CrossRef]

Valavanidis, A.; Vlachogianni, T.; Fiotakis, C. 8-hydroxy-2′-deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. *J. Environ. Sci. Health Pt. C-Environ. Carcinog. Ecotoxicol. Res.* 2009, 27, 120–139. [CrossRef]

LeBel, C.P.; Ischiropoulos, H.; Bondy, S.C. Evaluation of the probe 2’,7’-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. *Chem. Res. Toxicol.* 1992, 5, 227–231. [CrossRef]

Benzie, I.F.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Anat. Biochem.* 1996, 239, 70–76. [CrossRef]

Valavanidis, A.; Vlachogianni, T.; Fiotakis, C. 8-hydroxy-2′-deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. *J. Environ. Sci. Health Pt. C-Environ. Carcinog. Ecotoxicol. Res.* 2009, 27, 120–139. [CrossRef]

LeBel, C.P.; Ischiropoulos, H.; Bondy, S.C. Evaluation of the probe 2’,7’-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. *Chem. Res. Toxicol.* 1992, 5, 227–231. [CrossRef]

Benzie, I.F.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Anat. Biochem.* 1996, 239, 70–76. [CrossRef]

Valavanidis, A.; Vlachogianni, T.; Fiotakis, C. 8-hydroxy-2′-deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. *J. Environ. Sci. Health Pt. C-Environ. Carcinog. Ecotoxicol. Res.* 2009, 27, 120–139. [CrossRef]

LeBel, C.P.; Ischiropoulos, H.; Bondy, S.C. Evaluation of the probe 2’,7’-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. *Chem. Res. Toxicol.* 1992, 5, 227–231. [CrossRef]

Benzie, I.F.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Anat. Biochem.* 1996, 239, 70–76. [CrossRef]

Valavanidis, A.; Vlachogianni, T.; Fiotakis, C. 8-hydroxy-2′-deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. *J. Environ. Sci. Health Pt. C-Environ. Carcinog. Ecotoxicol. Res.* 2009, 27, 120–139. [CrossRef]
37. Vieira, C.E.D.; Pérez, M.R.; Acayaba, R.D.A.; Raimundo, C.C.M.; dos Reis Martinez, C.B. DNA damage and oxidative stress induced by imidacloprid exposure in different tissues of the Neotropical fish Prochilodus lineatus. Chemosphere 2018, 195, 125–134. [CrossRef]
38. Li, X.; Xiong, D.; Ding, C.; Fan, Y.; Ma, X.; Wang, C.; Xiong, Y.; Jiang, X. Exposure to water-accommodated fractions of two different crude oils alters morphology, cardiac function and swim bladder development in early-life stages of zebrafish. Chemosphere 2019, 235, 423–433. [CrossRef] [PubMed]
39. SAC. GB 17378.4-2007 The specification for marine monitoring—Part 4: Seawater analysis. In Standardization Administration of the People's Republic of China; Standards Press of China: Beijing, China, 2007; Volume GB 17378.4-2007, pp. 44–45.
40. EPA. Method 610: Polycyclic Aromatic Hydrocarbons; U.S. Environmental Protection Agency: Washington, DC, USA, 1984; p. 25.
41. EPA. Method 3510C: Separatory Funnel Liquid-liquid Extraction; U.S. Environmental Protection Agency: Washington, DC, USA, 1996; p. 8.
42. EPA. Method 3630C: Silica gel Cleanup, Part of Test Methods for Evaluating Solid Waste, Physical/Chemical Methods; U.S. Environmental Protection Agency: Washington, DC, USA, 1996; p. 15.
43. ISO. Water quality—Determination of 16 polycyclic aromatic hydrocarbons (PAH). In Water—Method Using Gas Chromatography with Mass Spectrometric Detection (GC-MS); International Organization for Standardization: Geneva, Switzerland, 2011; p. 24.
44. National Academies of Sciences Engineering and Medicine. The Use of Dispersants in Marine Oil Spill Response; The National Academies Press: Washington, DC, USA, 2019; p. 410.
45. Laramore, S.; Krebs, W.; Garr, A. Effects of exposure of pink shrimp, Farfantepeneaus duorarum, larvae to Macondo Canyon 252 crude oil and the Corexit dispersant. J. Mar. Sci. Eng. 2016, 4, 24. [CrossRef]
46. Rabeh, I.; Telahigue, K.; Bejaoui, S.; Hajji, T.; Chouba, L.; El Cafsi, M.h.; Soudani, N. Effects of mercury graded doses on redox status, metallothionein levels and genotoxicity in the intestine of sea cucumber Holothuria forskali. Chem. Ecol. 2019, 35, 204–218. [CrossRef]
47. Cuypers, A.; Karen, S.; Jos, R.; Kelly, O.; Els, K.; Tony, R.; Nele, H.; Nathalie, V.; Suzy, V.S.; Frank, V.B.; et al. The cellular redox state as a modulator in cadmium and copper responses in Arabidopsis thaliana seedlings. J. Plant Physiol. 2011, 168, 309–316. [CrossRef]
48. Sardi, A.E.; Renaud, P.E.; Morais, G.C.; Martins, C.C.; da Cunha Lana, P.; Camus, L.E. Effects of an in situ diesel oil spill on oxidative stress in the clam Anomalocardia flexuosa. Environ. Pollut. 2017, 230, 891–901. [CrossRef]
49. Ghiselli, A.; Serafini, M.; Natella, F.; Scaccini, C. Total antioxidant capacity as a tool to assess redox status: Critical view and experimental data. Free Radical Biol. Med. 2000, 29, 1106–1114. [CrossRef]
50. Ozhan, K.; Zahraeifard, S.; Smith, A.P.; Bargu, S. Induction of reactive oxygen species in marine phytoplankton under crude oil exposure. Environ. Sci. Pollut. Res. 2015, 22, 18874–18884. [CrossRef]
51. Schieber, M.; Chandel, N.S. ROS function in redox signaling and oxidative stress. Curr. Biol. 2014, 24, R453–R462. [CrossRef]
52. Tairova, Z.; Frantzen, M.; Mosbech, A.; Arukwe, A.; Gustavson, K. Effects of water accommodated fraction of physically and chemically dispersed heavy fuel oil on beach spawning capelin (Mallotus villosus). Mar. Environ. Res. 2019, 147, 62–71. [CrossRef]
53. Hodson, P.V. The toxicity to fish embryos of PAH in crude and refined oils. Arch. Environ. Contam. Toxicol. 2017, 73, 12–18. [CrossRef]
54. Incardona, J.P. Molecular mechanisms of crude oil developmental toxicity in fish. Arch. Environ. Contam. Toxicol. 2017, 73, 19–32. [CrossRef] [PubMed]
55. Patri, M.; Padmini, A.; Babu, P.P. Polycyclic aromatic hydrocarbons in air and their neurotoxic potency in association with oxidative stress: A brief perspective. Ann. Neurosci. 2010, 16, 22–30. [CrossRef]
56. Han, J.; Won, E.-J.; Hwang, D.-S.; Shin, K.-H.; Lee, Y.S.; Leung, K.M.-Y.; Lee, S.-J.; Lee, J.-S. Crude oil exposure results in oxidative stress-mediated dysfunctional development and reproduction in the copepod Tigriopus japonicus and modulates expression of cytochrome P450 (CYP) genes. Aquat. Toxicol. 2014, 152, 308–317. [CrossRef] [PubMed]
57. Almeda, R.; Cosgrove, S.; Buskey, E.J. Oil spills and dispersants can cause the initiation of potentially harmful dinoflagellate blooms (“red tides”). Environ. Sci. Technol. 2018, 52, 5718–5724. [CrossRef] [PubMed]
58. Gao, X.; Ding, G.; Li, X.; Xiong, D. Comparison of toxicity effects of fuel oil treated by different dispersants on marine medaka (Oryzias melastigma) embryo. *Acta Oceanol. Sin.* **2018**, *37*, 123–132. [CrossRef]

59. Afshar-Mohajer, N.; Fox, M.A.; Koehler, K. The human health risk estimation of inhaled oil spill emissions with and without adding dispersant. *Sci. Total Environ.* **2019**, *654*, 924–932. [CrossRef]

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