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The Red Tide Organism *Chaetoceros* sp. Responding to Exposure to Oil and Dispersant

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Abstract: Laboratory experiments were conducted to study the effects of oil spills and dispersants on the growth of the red tide organism *Chaetoceros* sp. Crude oil produced from the Chinese Bohai Sea, diesel oil, and the chemical dispersant (GM-2) produced in China were added into *Chaetoceros* sp. cultures. The results showed that both crude oil and diesel oil could enhance the growth of *Chaetoceros* sp. Data were analyzed by one-way ANOVA, and the confidence interval was 95%. At a concentration of 20 mg L^{-1} crude oil and a concentration of 10 µL L^{-1} diesel oil, *Chaetoceros* sp. bloomed to 1.57×10^5 cells mL^{-1} ($p < 0.01$) at day 14 and 3.55×10^4 cells mL^{-1} ($p < 0.05$) at day 10, respectively. A concentration of 10 µL L^{-1} diesel oil stimulated the specific growth rate for *Chaetoceros* sp. of 0.49 d^{-1} over 10 days. The specific growth rate of *Chaetoceros* sp. with 20 mg L^{-1} crude oil alone was 0.46 d^{-1} over 14 days. However, the mixture of oil and dispersant did not enhance the growth of *Chaetoceros* sp. as significantly as oil alone. These results implied that oil spills in coastal waters can stimulate *Chaetoceros* sp., and the specific dispersant GM-2 applied following oil spills may be unlikely to further enhance the growth of *Chaetoceros* sp.

Keywords: oil spill pollution; harmful algal bloom; chemical dispersants; emergency treatment

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1. Introduction

Oil pollution mainly results from accidents occurring during human activities such as shipping and oil exploitation. These accidents lead to millions of tons of petroleum hydrocarbons entering the ocean [1]. From 2000 to 2019, there were approximately 1–4 large spills (>700 tons) and 2–3 medium spills (7–700 tons) of crude oil into the seawater worldwide per year [2]. Oil spills in marine environment are extremely dangerous to the marine and coastal ecosystems [3]. In recent years, the oil spills that occurred in the Gulf of Mexico of the United States and in the Bohai Sea of China aroused attention concerning the pollution of petroleum hydrocarbons in the ocean [4]. There are about 100 wharves along the Bohai Sea coast. The Bohai Sea is very rich in oil and gas resources, for example, the Victory, Dagang, and Liaohe oil fields. These offshore oil fields have been assembled into one, becoming China's second largest oil field. Oil spill pollution is a serious threat to the Bohai Sea, and its ecology has become a focus of attention [5]. The petroleum-related human activities in the Bohai Sea have become potential risk factors for oil spills.

After oil spill accidents, applying dispersants is a common chemical method used to mitigate oil pollution. Dispersion breaks oil slicks into small droplets that are then mixed into the water column. Removal of the oil from the water surface may reduce contamination of the coast [6]. Since the *Torrey Canyon* oil spill, dispersants have been applied in the United States approximately 20 times and are routinely used internationally [7], including during the 1979 Ixtoc-I spill and the 2009 Montara spill [8]. However, the use of oil dispersants also poses potential secondary pollution to the marine ecosystem. Both petroleum hydrocarbons

and dispersants themselves have a certain toxicity [9]. Hence, the effect of dispersants on the marine environment caused by the oil spill should be assessed.

During the past several decades, the increasing outbreak frequency of red tides suggests a possible link to coastal pollution. It has been noted that red tide events increase as coastal pollution worsens [10]. Current studies have shown that low concentrations of petroleum hydrocarbons can stimulate the growth of marine microalgae [11,12], and dispersants can diffuse petroleum hydrocarbons into micron-sized small oil droplets [13,14]. In the presence of petroleum hydrocarbons, the growth rate of *Chaetoceros* sp. was altered by influencing the rates of CO_2 uptake, cell division, photosynthetic rate, and respiratory rate. The content, such as the chlorophyll-a in the cell, glycolipid, and triglyceride, may also change [15]. In addition, oil spills and dispersants can act as disrupters of predator–prey dynamics in plankton food webs and as indirect inducers of potentially harmful dinoflagellate blooms [9]. In addition to experimental methods, the relationship between oil spills and the occurrence of red tides has been explored through statistical analysis and multiple regression models. The frequency of red tides is positively correlated with the number of oil spills and the volume of oil spilled. The higher percentage of small spills (<7 tons) are more likely to enhance the outbreaks of red tides [16]. It has been suggested that there is a close relationship between oil spills and red tides.

Studies have shown that the Bohai Sea oil spill caused abnormal chlorophyll concentration distributions and red tides to nearby areas [5]. In addition, the number of studies on the effects of oil spills on marine phytoplankton has gradually been increasing. Studies have shown that 18 species of red tide Pyrrophyta and red tide Bacillariophyta were identified from 2000 to 2016, and Pyrrophyta appeared 39 times in the recorded red tide events in the Bohai Sea [17]. Many researchers have performed experiments on petroleum hydrocarbon as a factor in Pyrrophyta red tides [18]. To our knowledge, red tides with Bacillariophyta as the dominant species occur frequently in China and the *Chaetoceros* sp. is one of the common red tides' Bacillariophyta. However, the effects of oil and dispersant-treated oil on the growth and outbreaks of red tides from *Chaetoceros* sp. have seldom been studied. At the same time, different origins of oil and different brands and components of dispersants can influence red tides [12,19,20]. The Chinese Bohai Sea oil spills were characterized by crude oil from offshore oil drilling fields and diesel oil from ships. The latest critical oil spill causing devastating economic loss of aquaculture in the Chinese Bohai Sea was the accident at the Penglai 19-3 oil drilling platform that happened in 2011 [21]. Dispersants such as GM-2 have been widely used by the local governments to combat oil spills on-site in the Bohai Sea. Some research concerning the use of dispersants has shown that dispersants such as Corexit 9500 triggered the algal bloom of *Prorocentrum texanum* [9]. However, there is less research on the red tide species *Chaetoceros* sp. exposed to oil spills and the specific GM-2 dispersants. Our study thus has made an initial effort to answer the following two questions:

(1) What are the effects of local crude oil and diesel oil on the red tides species *Chaetoceros* sp.?
(2) Does the dispersant GM-2, used following an oil spill, further enhance the algal blooms of *Chaetoceros* sp.?

2. Materials and Methods

2.1. Materials

The test oil used in this study was crude oil produced in the Dongying Oil drilling fields of Shangdong, Bohai Sea (Figure 1). The diesel oil was obtained from the China National Petroleum Corporation (CNPC). The dispersant (GM-2) was produced by the Qingdao GuangMing Environmental Technology Company. This type of dispersant has been approved by the Maritime Safety Administration (MSA) of China for wide and domestic use in the oil spill contingency combat. The seawater for experiments was collected from "Huanghai Mingzhu" at Yantai with a salinity of 33.90. The seawater was collected and was settled for one week prior to use for removing impurities. The

Chaetoceros sp. were obtained from previous pure laboratory cultures. The *Chaetoceros* sp. were cultured in f/2-Si media [22] in an illumination incubator until the logarithmic growth phase required for subsequent experiments was met. The culture conditions simulated the environmental conditions during the summer red tide outbreak, set at 25 °C, a 14 h:10 h alternating cycle of light: dark, and an illumination intensity of 100 μmol m^{-2} s^{-1} [23]. Shaking the culture bottles manually once a day prevented algae from attaching to the bottle walls. There were no bubbled devices in the experiment.

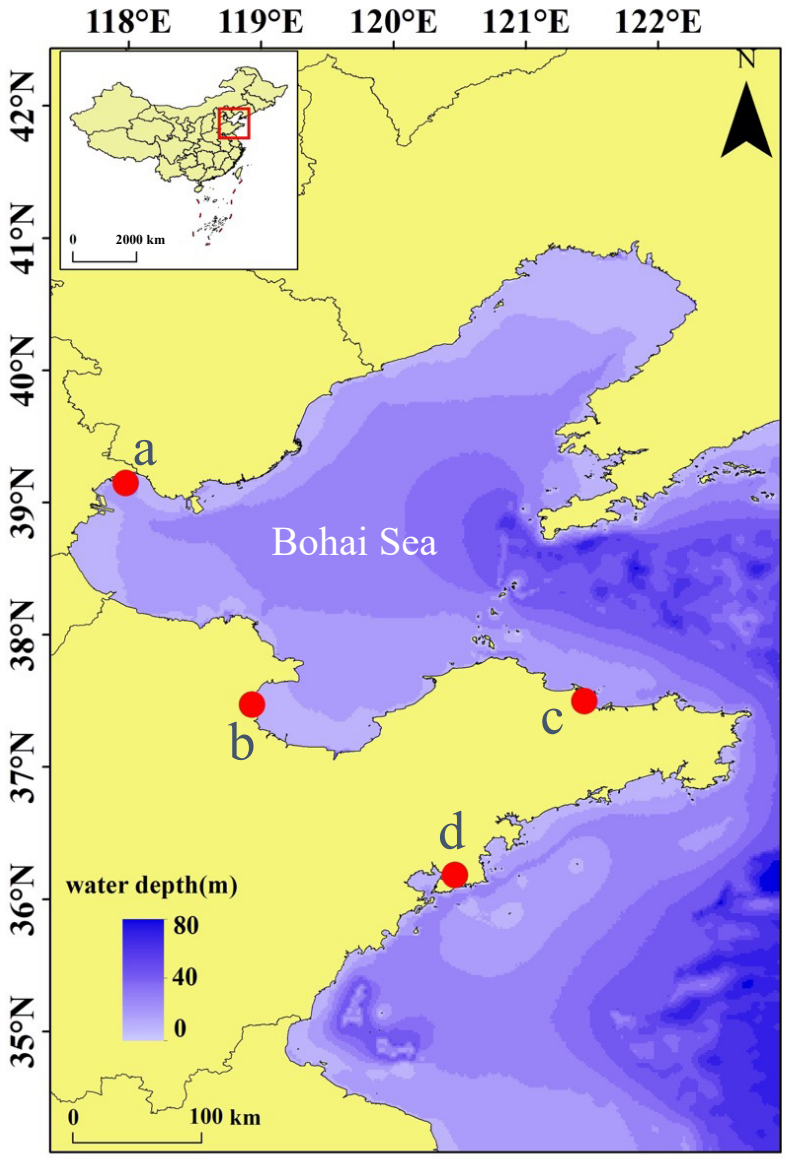

Figure 1. Origins of experimental materials from the Chinese Bohai Sea. Sites marked with red dots are (**a**) the *Chaetoceros* sp. red tide which occurred in 2012 [24]; (**b**) the origin of crude oil from the oil field; (**c**) the collection site of the seawater, and (**d**) the production and sale site of dispersants.

2.2. Experimental Setup and Observations

To prepare dispersant-treated oil, we used a ratio of dispersant to oil of 1:20 as suggested by Almeda et al. [21]; this was consistent with the range (1:10–1:50) recommended by the United States Environmental Protection Agency [25].

All experiments were conducted in a constant temperature-light incubator that simulated light and temperature conditions for *Chaetoceros* sp. growth. The algal seeds were transferred into 300 mL sterile seawater when the culture medium was activated for the experiments. The experiments were performed in duplicate. The experiments consisted

of different treatments (see Table 1): (1) with crude oil alone, (2) with dispersant alone, (3) with a mixture of crude oil and the dispersant, (4) with diesel oil alone, (5) with a mixture of the diesel oil and the dispersant, and (6) a blank control group (control: sterile sea water only). The initial abundance of *Chaetoceros* sp. for all experiments was set at about 150 cells mL^{-1}. The crude oil exposure concentrations in the experimental group were 5 mg L^{-1}, 10 mg L^{-1}, 20 mg L^{-1}, 50 mg L^{-1}, and 100 mg L^{-1}, concentrations consistent with the study by Almeda, et al. [26]. The diesel oil exposure concentrations in the experimental group were 20 µL L^{-1} and 40 µL L^{-1}. All culture media were sterilized by ultraviolet irradiation for 45 min. A short period of UV irradiation was employed to avoid possible photochemical changes of the diesel oil, crude oil, and the dispersant [27]. The experiments consisted of 14-day and 10-day laboratory incubations of *Chaetoceros* sp. exposed to crude oil and diesel oil treatments. The 14-day and 10-day incubation periods were determined by a series of preliminary test results.

Table 1. Overview of the conducted experiments with various settings.

Experiment	Treatment Name	Concentration	Duration
Exp.1 Crude oil	Control	0 (mg L^{-1})	14 days
	Crude oil	5 (mg L^{-1}) 20 (mg L^{-1}) 50 (mg L^{-1}) 100 (mg L^{-1})	
	Dispersant	0.25 (mg L^{-1}) 1.0 (mg L^{-1})	
	Mixture (Crude oil + Dispersant)	5 (mg L^{-1}) + 0.25 (mg L^{-1}) 20 (mg L^{-1}) + 1.0 (mg L^{-1})	
Exp.2 Diesel oil	Control	0 (µL L^{-1})	10 days
	Diesel oil	20 (µL L^{-1}) 40 (µL L^{-1})	
	Dispersant	1 (µL L^{-1}) 2 (µL L^{-1})	
	Mixture (Diesel oil + Dispersant)	20 (µL L^{-1}) + 1 (µL L^{-1}) 40 (µL L^{-1}) + 2 (µL L^{-1})	

Samples (1 mL) from each bottle were taken at regular intervals and fixed with Lugol's solution (1%) to determine changes in cell abundance during the incubation period. The cell concentration in each sample was determined using a 0.1 mL plankton counting chamber under an inverted microscope (LEICA DM500) at 40× magnification, and changes in cell morphology were observed.

2.3. Calculation and Statistical Methods

Origin 8.0 was used for mapping and SPSS 13.0 was used for data processing. The data were analyzed by one-way ANOVA. Analyzed by $p < 0.05$ indicates significant difference between data, $p < 0.01$ indicates an extremely significant difference. The average growth rate (μ) of *Chaetoceros* sp. can be calculated by the following formula [28]:

$$\mu_{i-j} = \frac{\ln X_j - \ln X_i}{t_j - t_i}$$

The $\mu_{i\text{-}j}$ is the average growth rate from t_i to t_j. X_i and X_j are the cell densities (cells mL^{-1}) at t_i and t_j, respectively.

3. Results

3.1. Effect of Crude Oil

The effect of crude oil on the growth of *Chaetoceros* sp. for 14 days is shown in Figure 2a. In the laboratory study with *Chaetoceros* sp., we found that this species grew exponentially and reached the bloom level > 5×10^3 cells mL^{-1} (i.e, the standard level of cell concentration forming a red tide = 5×10^3 cells mL^{-1}) in all treatments with crude oil pollutants after six days. Under the conditions of 5 mg L^{-1} and 20 mg L^{-1}, the stimulus effect on *Chaetoceros* sp. growth was clear. At 20 mg L^{-1}, the concentration of *Chaetoceros* sp. cells reached the maximum of more than 1.57×10^5 cells mL^{-1} at the 14th day, far more than the 5.6×10^4 cells mL^{-1} in the control group ($p < 0.01$) [28]. However, 50 mg L^{-1} and 100 mg L^{-1} of crude oil inhibited *Chaetoceros* sp. growth, as algae in those treatments reached the maximal algal concentrations of 2.73×10^4 cells mL^{-1} and 1.77×10^4 cells mL^{-1} in the culture process on days 8 and 14, respectively. *Chaetoceros* sp. growth under exposure to either 50 mg L^{-1} or 100 mg L^{-1} was relatively slow compared with the control group after day 8. These results showed that different exposure levels of crude oil have varied effects on the growth of *Chaetoceros* sp.

The concentrations of *Chaetoceros* sp. in the all mixed groups reached the level of red tide outbreak on the 4th day of culture (Figure 2b). The concentrations of algal cells in the crude oil and dispersant mixed groups were higher than that in the control group on the 6th day and the 8th day. In the mixture of 5 mg L^{-1} oil and 0.25 mg L^{-1} dispersant, the growth trend of *Chaetoceros* sp. was similar to but slower than the trend of the control group from the 8th day. On the 14th day of culture, the concentration of *Chaetoceros* sp. was 5.6×10^4 cells mL^{-1} in the control group; the concentration of *Chaetoceros* sp. in the mixture of 5 mg L^{-1} crude oil and 0.25 mg L^{-1} dispersant was 3.67×10^4 cells mL^{-1}. Both were lower than that in the control group (i.e., 5.6×10^4 cells mL^{-1}). However, from day 10 to day 14, the growth stimulus effect of *Chaetoceros* sp. cultured in the mixture of 20 mg L^{-1} oil and 1 mg L^{-1} dispersant was significant, as the concentration reached 9.43×10^4 cells mL^{-1} on the 14th day, which was far more than that in the control group (i.e., 5.6×10^4 cells mL^{-1}). Apparently, the mixture of 20 mg L^{-1} oil and 1 mg L^{-1} dispersant was more suitable for *Chaetoceros* sp. growth.

The effects of dispersants with varied exposure levels on the growth of *Chaetoceros* sp. are shown in Figure 2c. In the first four days of culture, the addition of dispersant had no major effect on the growth of *Chaetoceros* sp. From day 4 to day 8, 0.25 mg L^{-1} dispersant enhanced the growth of *Chaetoceros* sp. The concentration of *Chaetoceros* sp. reached 2.97×10^4 cells mL^{-1} on the 8th day. This was higher than that of the control group (i.e., 1.7×10^4 cells mL^{-1}). However, the concentration of *Chaetoceros* sp. in the treatment of 0.25 mg L^{-1} dispersant was consistently lower than in the control group from the 8th day. On the 14th day of culture, the concentration of *Chaetoceros* sp. reached 2.87×10^4 cells mL^{-1}. It is worth noting that compared with the control group, 1.0 mg L^{-1} dispersant significantly stimulated the growth of *Chaetoceros* sp. ($p < 0.05$) from day 12 to day 14. On day 14, the concentration of *Chaetoceros* sp. had reached 8.03×10^4 cells mL^{-1}, which was 1.4 times higher than in the control group (i.e., 5.6×10^4 cells mL^{-1}). Moreover, this concentration was 2.8 times higher than the 0.25 mg L^{-1} in the dispersant group (i.e., 2.87×10^4 cells mL^{-1}).

We selected the experimental group data with the most significant stimulating effect on the growth of algae to calculate the specific growth rate of *Chaetoceros* sp. (Figure 3). The differences of the specific growth rate among the groups were analyzed by ANOVA. The specific growth rate of *Chaetoceros* sp. in the crude oil alone was significantly higher than in the control treatment and the mixtures. The specific growth rate of crude oil alone was 0.46 d^{-1}, while those of the control group and the mixture treatment group were 0.35 d^{-1} ($p < 0.01$) and 0.36 d^{-1} ($p < 0.01$), respectively. Moreover, there was no statistically significant difference between the growth rate in the mixture group and that in the control group ($p > 0.05$). The results demonstrated that the crude oil treated with dispersant was more

toxic to *Chaetoceros* sp., so that the dispersant-treated crude oil could not enchance further the growth of *Chaetoceros* sp. more than the crude oil alone could.

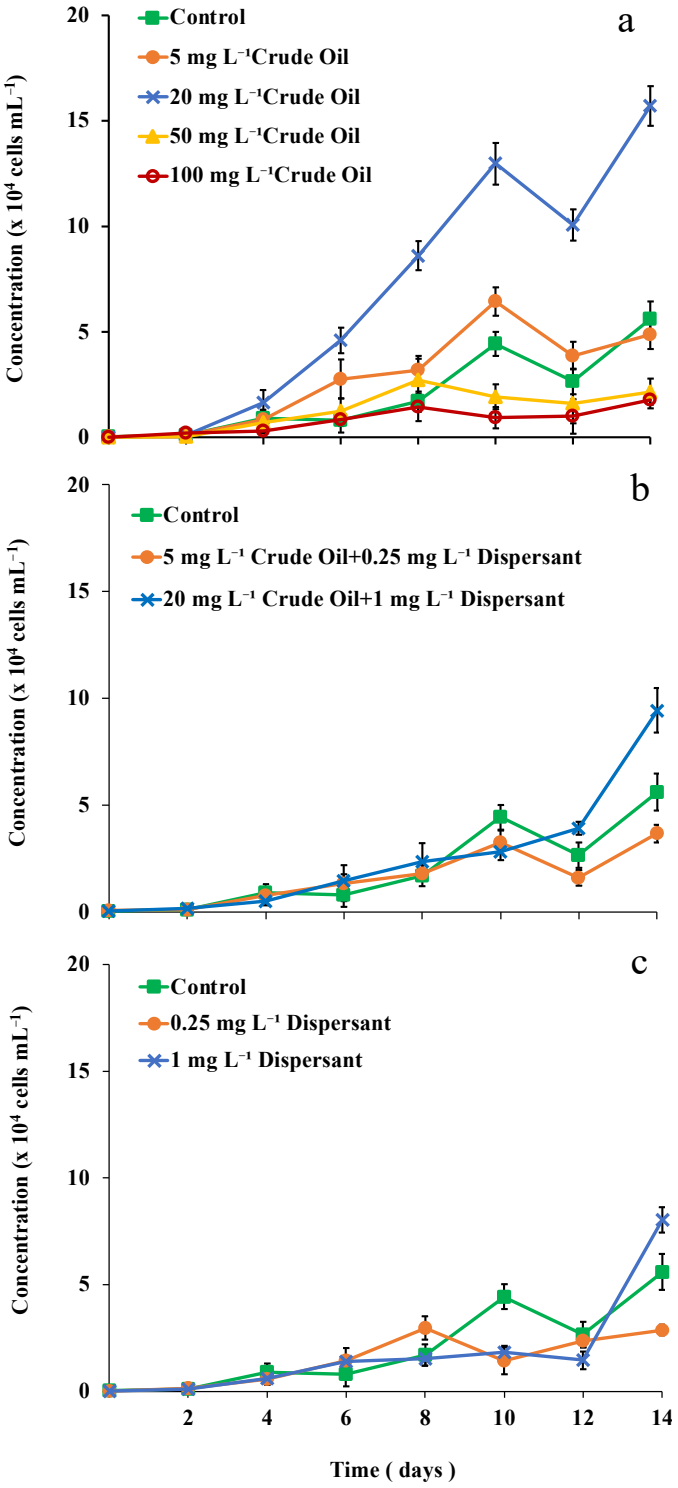

Figure 2. The growth curve (cells mL^{-1}) of *Chaetoceros* sp. during the 14-day incubation period following exposure to (**a**) crude oil alone, (**b**) a mixture of crude oil and dispersant, and (**c**) dispersant alone. The cell concentrations were observations of sampling at intervals after exposure to the specific pollutants during the incubation. The error bars are derived from calculations in parallel experiments. The error bars indicate the 95% confidence intervals.

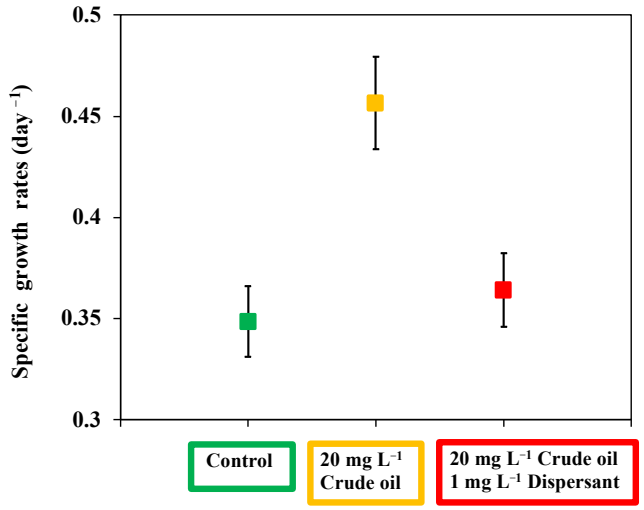

Figure 3. Specific growth rates (d^{-1}) of *Chaetoceros* sp. in the different treatments during the 14-day incubation period. The treatments included the absence of pollutants ("control"), crude oil alone (20 mg L^{-1}, "crude oil"), and dispersant-treated crude oil (20 mg L^{-1} crude oil and 1 mg L^{-1} dispersant, "crude oil + dispersant"). The criteria selected for the concentration of contamination were to achieve the maximum cell concentration in the treatment group (exceeding the red tide standard level of 5000 cells mL^{-1}). The error bars indicate the 95% confidence intervals.

3.2. Effect of Diesel Oil

The addition of different concentrations of diesel oil to the culture process of *Chaetoceros* sp. had various effects on the growth of the algae. Figure 4 shows the growth curves of *Chaetoceros* sp. within 10 days for the treatments of diesel oil alone, dispersant alone, and dispersant-treated diesel oil. As shown in Figure 4a, in the first 2 days of culture, diesel oil had no significant effect on the growth of the algae ($p > 0.05$). On the 4th day, the concentration of *Chaetoceros* sp. cells in the 10 μL L^{-1} diesel oil group was 1.58×10^4 cells mL^{-1}, and it was higher than the control (1.33×10^4 cells mL^{-1}). However, in the 20 μL L^{-1} and 40 μL L^{-1} diesel oil treatment group, there were 9.38×10^3 cells mL^{-1} and 8.03×10^3 cells mL^{-1}, respectively. Moreover, *Chaetoceros* sp. treated with 10 μL L^{-1} diesel oil continued to increase up to 10 days and peaked at 3.55×10^4 cells mL^{-1} on the 10th day, it was much higher than the control (2.22×10^4 cells mL^{-1}). However, the numbers of algal cells in the 20 μL L^{-1} and 40 μL L^{-1} diesel oil treatment group were always lower than that in the control group from the 2nd day. On the 10th day, their maximum values were 1.08×10^4 cells mL^{-1} and 1.05×10^4 cells mL^{-1}, respectively. The results demonstrated that the exposure to 10 μL L^{-1} diesel oil significantly stimulated the growth of *Chaetoceros* sp. (Figure 4a, $p < 0.05$). By the 10th day, the maximal algal cell concentration of *Chaetoceros* sp. (i.e., 3.55×10^4 cells mL^{-1}) in the 10 μL L^{-1} diesel oil treatment was nearly 7.1 times greater than the standard level of a red tide (i.e., 5×10^3 cells mL^{-1}).

Overall, in the experiments with the mixture of diesel oil and dispersant (Figure 4b), the number of cells in the mixture pollutants was lower throughout than in the control group. The maximum concentration of algal cells in the control group was 2.22×10^4 cells mL^{-1} by the 10th day, which was higher than that in other experimental groups with the mixture pollutants added. It can be seen that the concentration of mixed pollutants in the experiment has an inhibitory effect on the growth of algae. I'm sorry that I didn't quite understand this comment, which is consistent with the data in the figure. So I just made a little change, and if there are any problems, I will continue to make changes.

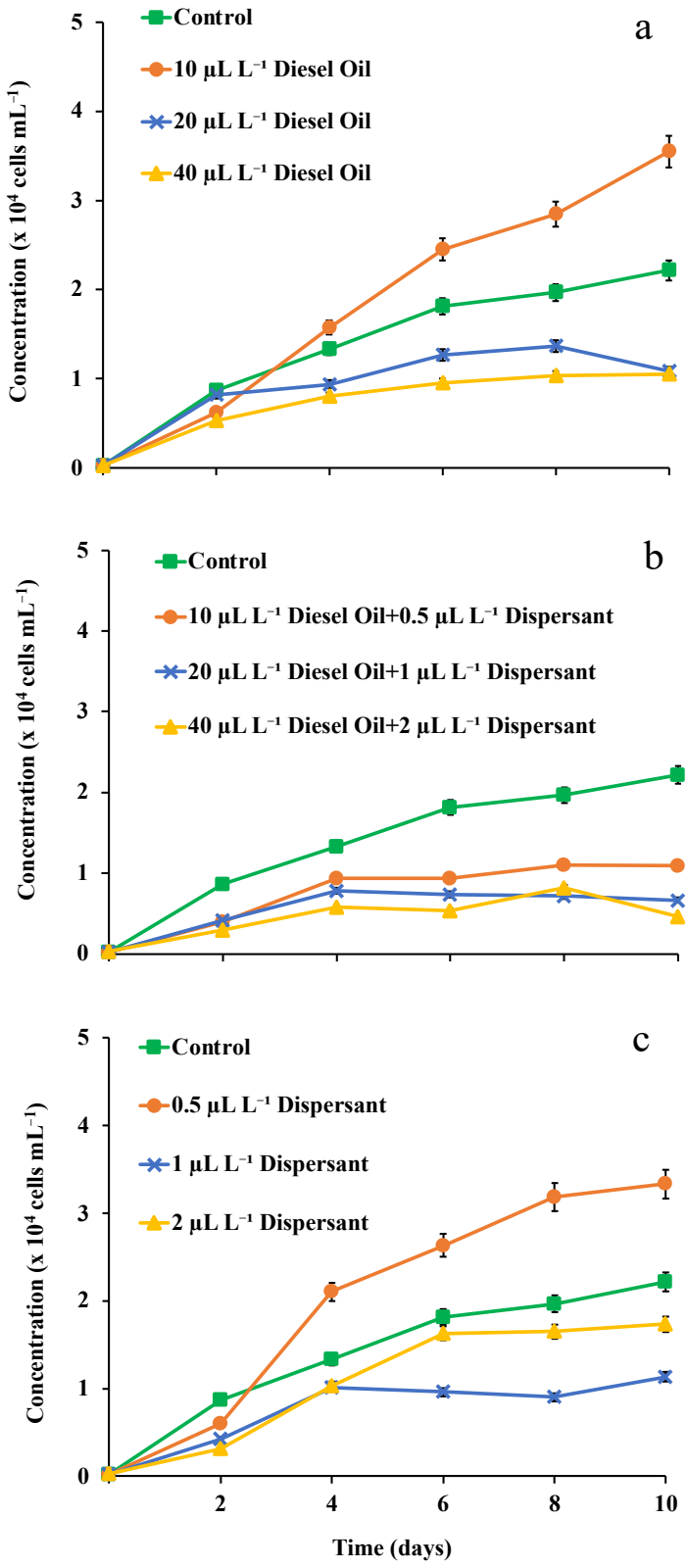

Figure 4. The growth curve (cells mL^{-1}) of *Chaetoceros* sp. during the 10-day incubation period following exposure to (**a**) diesel oil, (**b**) a mixture of diesel oil and dispersant, and (**c**) dispersant alone. The cell concentrations are the results of sampling at intervals after exposure to the studied pollutants during a single culture. The error bars are derived from calculations in parallel experiments. The error bars indicate the 95% confidence intervals.

During the 10 days, the growth of *Chaetoceros* sp. following the addition of 0.5 μL L^{-1} dispersant gradually increased compared with the control group (Figure 4c). On the 10th day, the maximum algal cells in the 0.5 μL L^{-1} dispersant was 3.33 × 10^4 cells mL^{-1}, and it was higher than the control (2.22 × 10^4 cells mL^{-1}). However, in the 1 μL L^{-1} and 2 μL L^{-1} dispersant groups, the concentrations of algal cells were 1.13 × 10^4 cells mL^{-1} and 1.73 × 10^4 cells mL^{-1}, which were the highest in the entire experimental cycle on day 10. Therefore, *Chaetoceros* sp. in 0.5 μL L^{-1} dispersant had a significant stimulus ($p < 0.05$), but the higher concentrations of dispersant had an inhibitive effect on *Chaetoceros* sp.

The specific growth rates in varied treatment groups within 10 days of culturing are shown in Figure 5. The growth rate of *Chaetoceros* sp. treated with 10 μL L^{-1} diesel oil was 0.49 d^{-1}, higher than the 0.45 d^{-1} in the control group ($p < 0.05$). However, the specific growth rates of both 20 μL L^{-1} and 40 μL L^{-1} concentration diesel oil and dispersant-treated diesel oil were lower than those of the control group. The specific growth rate of 0.5 μL L^{-1} concentration dispersant was 0.49 d^{-1}, while that of 10 μL L^{-1} diesel and 0.5 μL L^{-1} dispersant mixture was 0.39 d^{-1}. It was showed that the addition of dispersant inhibited the stimulating effect of diesel oil on the growth of *Chaetoceros* sp.

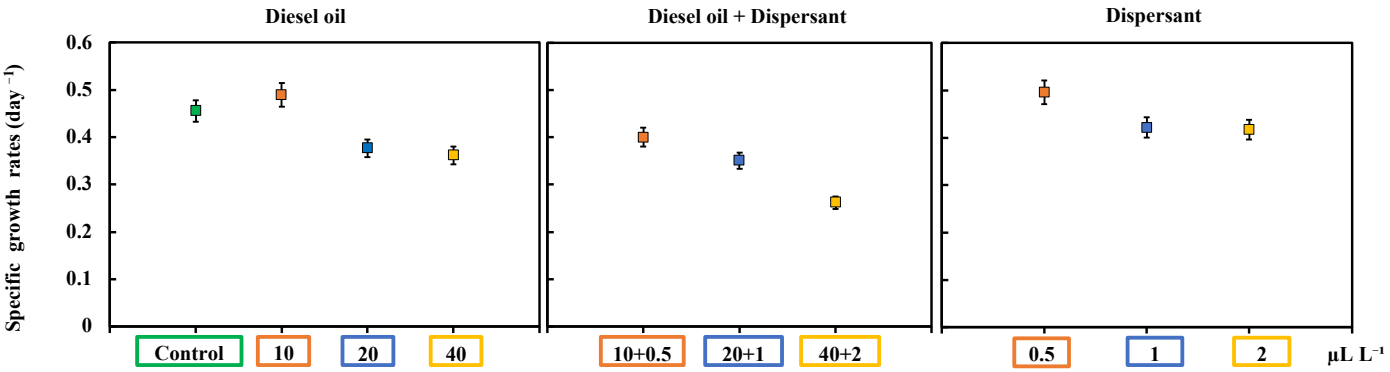

Figure 5. Specific growth rates (d^{-1}) of *Chaetoceros* sp. in the different treatments during the 10-day incubation period. The different treatments comprised the absence of pollutants ("control"), diesel oil alone (10 μL L^{-1} diesel oil, 20 μL L^{-1} diesel oil, 40 μL L^{-1} diesel oil), dispersant-treated diesel oil (10 μL L^{-1} diesel oil + 0.5 μL L^{-1} dispersant, 20 μL L^{-1} diesel oil + 1 μL L^{-1} dispersant, 40 μL L^{-1} diesel oil + 2 μL L^{-1} dispersant), and dispersant alone (0.5 μL L^{-1} dispersant, 1 μL L^{-1} dispersant, 2 μL L^{-1} dispersant). The error bars indicate the 95% confidence intervals.

4. Discussion

4.1. The Effect of Crude Oil

Our experimental results demonstrated *Chaetoceros* sp. tolerance to 50 mg L^{-1} crude oil during the first eight days of culturing, and its algal concentration surpassed that of the control group. In summary, 5 mg L^{-1}, 20 mg L^{-1}, and 50 mg L^{-1} of crude oil stimulated the growth of *Chaetoceros* sp. to the outbreak level of a red tide. Among the crude oil treated groups, 20 mg L^{-1} of crude oil appeared to be the most suitable concentration for algal growth, with a peak of 1.57 × 10^5 cells mL^{-1} on the 14th day. The reason for the growth of algal cells may be that petroleum hydrocarbons can affect the synthesis of proteins, nucleic acid, and other biological macromolecules in marine phytoplankton, and the synthesis rate directly reflects the metabolism and growth of the organism [4]. The study of El-Sheekh et al. showed that the protein content of green algae increased after treatment with crude oil less than 0.05% and lubricating oil less than 0.5% (mass fraction) [11]. As a result, algae accelerate the accumulation of proteins in their bodies to promote their ability to detoxify petroleum hydrocarbons. Previous studies were consistent with our findings; in the presence of crude oil, *Phaeodactylum tricornutum*, *Chaetoceros* sp., *Dunaliella* sp., and *Chlorella* sp. all grew larger and showed oil resistance in single cultures [29]. Under the effect of low concentration (1.5–6 g L^{-1}) of anthracene, the growth of *Skeletonema costatum* showed a clear "toxicant excitatory effect", and the cell density increased significantly [30].

Moreover, heterotrophic dinoflagellate algae were found to ingest crude oil, aiding the algae to enter the marine food web. At the crude oil concentration of 1 μL L^{-1} commonly found after an oil spill, the heterotrophic dinoflagellates such as *Noctiluca scintillans* and *Gyrodinium spirale* grew rapidly and ingested 0.37 μg-oil μg-$C_{dino}{}^{-1}$ d^{-1}, which could represent 17% to 100% of dispersed oil in surface waters when heterotrophic dinoflagellates are abundant or in bloom [31]. Therefore, we propose to test the hypothesis that *Chaetoceros* sp. is capable of ingesting less concentrated crude oil than heterotrophic dinoflagellates in future work.

Although low concentrations of petroleum hydrocarbons enhance the growth of phytoplankton, higher concentrations of petroleum hydrocarbons inhibit the growth of phytoplankton; this is known as the excitatory effect or stimulation effect of toxic substances. Crude oil stimulates algal growth in many ways. Previous studies have shown that under the stress of high petroleum hydrocarbon concentration, the photosynthetic rate of most phytoplankton decreases, and the respiration rate increases to adapt to the stress environment [4]. The inhibition of the photosynthesis of marine phytoplankton by petroleum hydrocarbons is mainly due to the accumulation of PAHs and other organic pollutants in the hydrophobic thylakoid membrane of phytoplankton, which damages the structure of the plasma membrane, breaks the balance between ions in the cell membrane, and interferes with electron transfer during photosynthesis. Besides, marine phytoplankton compensate for the loss of energy due to the inhibition of photosynthesis through enhanced respiration. In addition to changes in photosynthesis and respiration, petroleum hydrocarbons also affect phytoplankton enzyme activities. Aksmann et al. showed that under the action of 500 μg L^{-1} anthracene and 1.0 × 10^4 μg L^{-1} phenanthrene, the activity of superoxide dismutase in *Scenedesmus armatus* cells was 127–78% and 234–293% of the control, respectively [32]. It can be seen that a certain concentration of phenanthrene, anthracene, and other pollutants can stimulate the enzyme activity of antioxidant enzymes in algal cells, but if the pollutant exceeds a certain concentration, it will inhibit the activity of antioxidant enzymes in algal cells, and the concentration threshold of pollutants that produces inhibitory effect is related to the species of marine phytoplankton [4].In our laboratory experiments, the concentration of 100 mg L^{-1} crude oil showed a clear inhibitory effect on the growth of algae during the culture period of 14 days, always leading to a lower algal concentration than the control group. According to published data, crude oil with a mass fraction of 0.1% delayed the growth adaptation periods of *Nitzschia linearis* and *Scenedesmus obliquus*, reduced their total biomass, and caused morphological changes in both species [33].

The growth of *Chaetoceros* sp. was somewhat inhibited, and the adaptation period was prolonged in the mixed group compared with the crude oil alone. The rapid growth phase of *Chaetoceros* sp. started from the 2nd day in the treatment of 20 mg L^{-1} crude oil alone, but the algae began to grow rapidly in the mixture of 20 mg L^{-1} crude oil and 1 mg L^{-1} dispersant on the 4th day. The specific growth rates shown in Figure 3 support such a conclusion. The toxic effects of crude oil and dispersants from different sources on marine organisms are varied, and the tolerance of different tested organisms to crude oil and oil spill dispersants also varies widely. For example, Tang tested BP-1100X dispersant and found that the use of BP-1100X alone had a large effect on the stability of the population structure of the marine plankton, while the addition of the oil mixed with the dispersant had a lesser effect on the phytoplankton population [34]. In contrast with *Chaetoceros* sp., *Ulva pertusa* was observed to grow better in the mixture of dispersant (Shuangxiang-1) and crude oil than in the crude oil alone [35].

4.2. The Effect of Diesel Oil

Although the hydrocarbons in the diesel oil are different from those of the crude oil, the influence on *Chaetoceros* sp. was essentially the same, and was also basically consistent with the previous research results.

In previous studies, the concentration of diesel oil hydrocarbons from 0.1 to 10 mg $(dm^3)^{-1}$ showed a stimulus effect on the growth of *Chaetoceros* sp., and the stimulus effect increased initially and then decreased with the increase in diesel oil hydrocarbon concentration [15]. As the results of this study show, the growth stimulus effect of 10 μL L^{-1} diesel oil on *Chaetoceros* sp. was significant ($p < 0.05$). However, the diesel concentration of 20 μL L^{-1} and 40 μL L^{-1} had an inhibited effect on the growth of *Chaetoceros* sp. compared with 10 μL L^{-1} diesel oil. Although the exposure method used for diesel oil in this study is different from other studies, the significance and methodology of the study are similar.

The experimental results showed that the addition of dispersant (GM-2) increased the toxicity of diesel oil and reduced the specific growth rate of *Chaetoceros* sp. cells in a one-time culture. British light diesel oil was more toxic to *Chlorella halophytes* when mixed with dispersant [27]. This is also consistent with our findings. In addition, the concentration ratio of diesel oil to dispersant is an important factor to consider.

4.3. The Addition of Contaminants

Many studies have used the mother liquor of petroleum hydrocarbon aqueous solution [36]. In these experiments, the aqueous solution components of petroleum hydrocarbons were directly added to the habitats of phytoplankton as pollution sources. However, the short-term consequence of an oil spill is that oil enters the sea water, and it takes a long time for the components of an aqueous solution to form [37]. This study explored the effects of crude oil and dispersants on the growth curve of *Chaetoceros* sp. in the short period after a crude oil spill. Therefore, following Almeda, et al. [26], we added crude oil, diesel oil, and dispersant without treatment in the experiment to simulate the impact of crude oil leakage in the natural environment. This contaminant addition method has also been used in previous experiments using bioassay techniques to determine the ability of freshwater phytoplankton assemblages to degrade and clean up oil spills in the surrounding environment [33]. Diesel oil has a low viscosity and is convenient to be absorbed directly. Compared with crude oil, diesel oil is more water-soluble. Therefore, this study employed diesel oil and added it to the growth environment of the algae, and this addition method simulated the situation of a marine ship fuel oil leakage.

4.4. Limitations

We only studied the effects of oil and dispersant exposure on *Chaetoceros* sp. and did not explore the final concentration of dissolved components of crude oil in aqueous solution. We explored the immediate effects of an oil spill on *Chaetoceros* sp. in the short term. Further study is needed to investigate the long-term impacts of oil spills on the red tide organisms in a marine environment. At the same time, competition and symbiosis exist between other phytoplankton and *Chaetoceros* sp. in natural conditions. For example, *Chaetoceros curvisetus* has an allelopathic effect on *Skeletonema costatum* [38]. This relationship between different algae is also a factor that we need to consider in future in situ simulation experiments.

Only the commonly used ratio of 20 oil: 1 dispersant in dealing with oil spill accidents was selected in this study; the influence of different ratios was not explored.

Eventually, regarding the use of dispersants, it is not only necessary to understand the impact on a particular organism [39] but also to comprehensively examine the impact of dispersants and oil spill emulsions on the food chain and the entire ecosystem [40]. Therefore, more experimental studies are urgently needed to clarify the impact of dispersants on the ecosystem and to simulate the ecological environment under natural conditions as much as possible.

5. Conclusions

It has been demonstrated that an oil spill at a certain concentration can stimulate the growth of *Chaetoceros* sp., making it more likely to induce the formation of red tides. Empirical evidence showed that *Chaetoceros* sp. grew rapidly in 20 mg L^{-1} crude oil, up to 1.57×10^5 cells mL^{-1} at an average specific growth rate of 0.46 d^{-1} for the 14 days'

culturing, far exceeding the standard level of a red tide. Under the combined conditions of 20 mg L^{-1} crude oil and 1 mg L^{-1} dispersant, *Chaetoceros* sp. had an increasing trend and reached 9.43 × 10^4 cells mL^{-1} on the 14th day, which was much less than that of the crude oil alone group. At this time, compared with the mixture, crude oil alone had an extremely significant effect on the growth of *Chaetoceros* sp. ($p < 0.01$). At the diesel oil concentration of 10 μL L^{-1}, the specific growth rate of *Chaetoceros* sp. was 0.49 d^{-1} within 10 days, and the maximum abundance of algae reached 3.55 × 10^4 cells mL^{-1} on the 10th day. Diesel oil has stimulated effect on the growth of *Chaetoceros* sp. compared with the control ($p < 0.05$). In addition, we observed that the growth of *Chaetoceros* sp. in either oil (i.e., crude oil or diesel oil) treated with dispersant was not faster than that in either oil without dispersant. Therefore, from the point of view of oil spill cleanup strategy, oil spills in coastal waters may stimulate *Chaetoceros* sp., and the dispersant GM-2 applied following the oil spill is unlikely to enhance the bloom.

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