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Stronger impact of dispersant plus crude oil on natural plankton assemblages in short-term marine mesocosms

Seung Won Jung[a], Oh Youn Kwon[b], Chang Kyu Joo[c], Jung-Hoon Kang[b], Moonkoo Kim[c], Won Joon Shim[c], Young-Ok Kim[b,*]

[a] Library of Marine Samples, Korea Ocean Research and Development Institute, Geoje 656-830, Republic of Korea
[b] South Sea Environment Research Department, Korea Ocean Research & Development Institute, Geoje 656-830, Republic of Korea
[c] Oil and POPs Research Group, Korea Ocean Research & Development Institute, Geoje 656-830, Republic of Korea

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ABSTRACT

To assess the effects of crude oil and dispersant on marine planktonic ecosystems, analyses were performed in 1000-L mesocosm over a period of nine days. Triplicate experiments were conducted for two different treatments, namely, addition of crude oil alone and oil plus dispersant. In the mesocosm with oil plus dispersant, high concentrations of total petroleum hydrocarbon (TPH) were soon found in the bottom layer. In addition, most planktonic communities responded drastically to the presence of dispersant acting to disperse TPH: total bacterial abundances increased for the first two days and then decreased rapidly for the remainder of the experiment. The abundance of heterotrophic flagellates increased rapidly in association with the increase in bacterial cells. The abundance of phytoplankton and zooplankton communities decreased clearly within two days. Time-delayed relationship also revealed that the TPH concentration had a significant negative relationship with phyto- and zooplankton communities within two days. However, most planktonic communities were affected less adversely in the mesocosms treated with crude oil alone than in those treated with both crude oil and dispersant. The present results demonstrate that the planktonic ecosystem was damaged more severely by the introduction of dispersant than by the harmful effects of crude oil itself. Therefore, caution should be taken when considering the direct application of dispersant in natural environments, even though it has the advantage of rapidly removing crude oil.

1. Introduction

Spills of large quantities of crude oil have the potential to cause severe short- and long-term damage to marine ecosystems. In general, organisms that are injured chronically due to oil pollution are large, and include macrobenthos and fishes. On the other hand, small organisms such as bacteria, phytoplankton, and zooplankton are affected rapidly upon exposure to oil pollution [1,2]. Plankton form the foundation of food webs, and are the primary source of food for many macroscopic organisms. Over the last 30 years, although numerous studies have examined the effects of oil on planktonic communities [3], most studies have focused on the effects of exposure in the water-accommodated fraction (WAF) in laboratories or investigated variation in organisms within natural areas that have been affected by oil spills [4]. However, WAF tests have been of limited use in understanding the potential effects of oil exposure on ecosystems because interactions between biotic and abiotic factors in natural environments are very complex and the investigation of oil spills in natural ecosystems poses enormous logistical challenges, which include high costs and the need for long-term study. To overcome these problems, mesocosm studies are an effective approach to bridge the gap between information obtained from laboratory studies and the responses of organisms in ecosystems. In addition, mesocosm studies can improve our understanding of the impact of oil spills on ecosystems and possibly enable prediction of the effects of oil on entire ecosystems [5].

The use of chemical dispersants can be an effective method to remove crude oil at sea. These dispersants are capable of rapidly removing large amounts of certain types of oil from the sea surface and transferring it into the water column [6]. Despite the many studies related to the effects of oil that have been conducted over the past decade, researcher' opinions are diverse on the effectiveness of dispersants at sea, because the interplay of surfactants and solvents in commercial formulations of dispersant with crude oil is very complex [7,8].

On 7th December, 2007, 6.5 nautical miles off the coast of Taean, Southwest Korea, an estimated 12,547 kL (10,900 M/T) of three

* Corresponding author. Tel.: +82 55 639 8520; fax: +82 55 639 8509.
E-mail address: yokim@kordi.re.kr (Y.-O. Kim).

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different types of crude oil, namely, oil from the Upper Zakum oil field (UAE), Kuwait export crude, and Iranian heavy crude, were released after a collision between the oil tanker *M/V Hebei Spirit* and a barge carrying a crane. The spill led to the rapid spread of oil along the coastline of Taean owing to severe weather conditions, which included waves of up to 4 m and a prevailing north-westerly wind (10–14 m/s). More than 70 km of the coastline of Taean was impacted heavily by the spill, and natural marine communities and aquaculture facilities were destroyed. To remove the oil, approximately 298 tons of dispersant was released into the area and clean-up operations were initiated immediately after the spill [9]. The accident, which resulted in serious damage to the Korean coast, ranks alongside spills from the *Prestige* off the coast of Spain in 2002, the *Tasman Spirit* off the coast of Pakistan in 2003, and the *Solar 1* off the Philippines in 2006 as one of the largest tanker spills in recent years [10].

In a previous study, we introduced Iranian heavy crude oil at several concentrations of 10, 100, 1000, 5000 and 10,000 ppm (v/v) into a small-scale field microcosm and investigated whether it inhibited or stimulated the growth of microbial communities [11]. When the crude oil was added to the microcosm at a concentration higher than 1000 ppm (v/v), microbial communities changed dramatically that the growth of specific bacteria appeared to be stimulated. Although the results from this previous study using a microcosm suggest that assessments of the risks posed by oil pollution should consider the level of oil exposure in a specific situation, these results could not be extrapolated fully to marine planktonic ecosystems because we did not measure the effects of a dispersant, changes in petroleum hydrocarbon, or fluctuation in environmental factors and plankton communities. In addition, we did not carry out experiments on a large scale and throughout the water column. To address these limitations, we conducted a vertical mesocosm study and focused on interactions of planktonic communities and environmental factors upon exposure to the spilled crude oil and the dispersant. A possible scenario that describes the responses of biotic/abiotic factors to oil pollution is discussed.

2. Materials and methods

2.1. Experimental mesocosm setup

To evaluate the responses of planktonic communities to the introduction of crude oil and dispersant, nine marine vertical mesocosms were immersed in the water column at a site (34° 59′ 37.48″ N, 128° 40′ 27.53″ E) used by the South Sea Branch of the Korea Ocean Research and Development Institute located off the coast of Geoje Island, South Korea. Each cylinder-shaped mesocosm (0.5 m in diameter and 5 m in depth) comprised a 1200-L enclosure that contained 1000 L of seawater, and was made of a transparent polyethylene material reinforced with a polyester grid (Fig. 1). To supply identical masses of water to each mesocosm, a single body of seawater filtered through a net with a 200-μm mesh to eliminate large particle substances, including organisms that were mixed in a tank with a volume of 10,000 L was supplied slowly to all mesocosms. Zooplankton (>200 μm in size) were inoculated into each mesocosm at a density of 150 individuals/L, which is the same density found in natural waters, using a net with a 200-μm mesh. The water in the mesocosms was exposed to crude oil alone (Iranian heavy crude; the type of oil that was predominant among that spilled from the *Hebei Spirit*) at a concentration of 1000 ppm (v/v; hereafter, OIL group) or a mixture of crude oil and dispersant, namely, Hi-Clean (Daeil Chemical. Co., Korea; O + D group). Crude oil (1000 ppm; v/v) was dispensed directly onto the surface of the seawater, to form a slick. In the O + D group, 100 ppm (v/v) dispersant was then dispensed directly onto the slick, which gave a

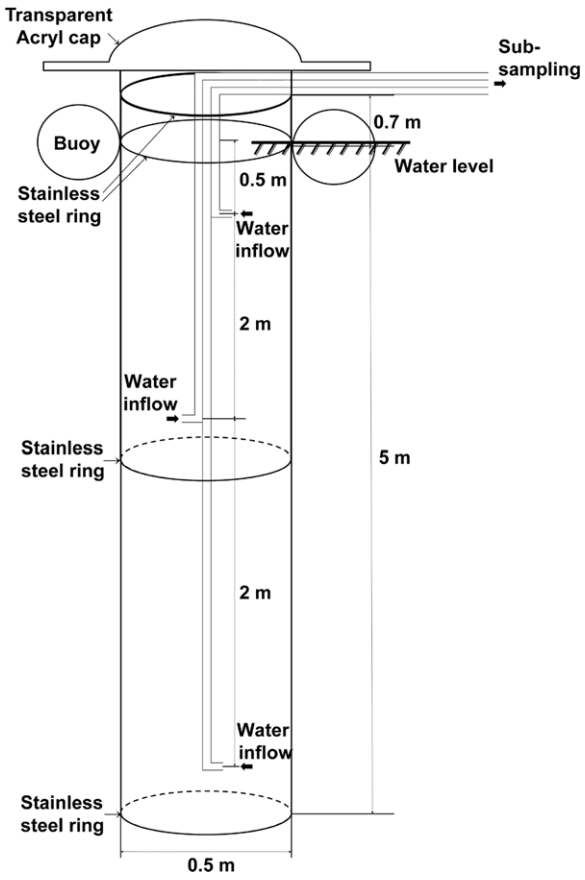

Fig. 1. Schematic diagram of the mesocosm used.

dispersant-to-oil ratio of 1:10. This was the dispersant-to-oil ratio that was used in general to disperse the oil spill formed by the *Hebei Spirit* accident. A control mesocosm, to which no oil or dispersant was added, was also prepared. In addition to natural wave action, the water in the mesocosms was mixed by artificial vertical mixing for 5 min twice a day. Each experiment, namely, OIL group, O + D group, and control group, was carried out in triplicate over nine days from 16 to 24 April, 2009.

2.2. Measurement of environmental factors and total petroleum hydrocarbons

Subsamples from the surface (depth of 0.5 m), middle (2.5 m), and bottom (4.5 m) of the water column were collected using a pump-based sampler with a Master Flex L/S peristaltic pump (Cole Parmer, USA), which minimized the risk of contamination from the layer of oil on the surface. Samples were taken daily from each mesocosm at 9:00 AM. Water temperature, pH, salinity, and dissolved oxygen (DO) content were measured immediately in the subsamples using a portable multi-parameter meter (556 MPS, YSI, USA) and light intensity was measured using a quantum meter (LI-189, Li-Cor, USA). To analyse inorganic nutrient concentrations [dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorus (DIP), and dissolved silica (DSi)], a 250-mL sample filtered through a 47-mm Whatman GF/F filter was stored in an acid-cleaned polyethylene (PE) bottle at −80 °C. Nutrient concentrations were analysed using a nutrient auto-analyser (Lachat Quickchem, Lachat Instruments, USA). To analyse chlorophyll *a* concentrations, a 250-mL sample was filtered through a GF/F filter under low vacuum pressure. The filter was then soaked in 15 mL of cold 90% acetone-distilled water (v/v), sonicated to break cell walls, and

Table 1
Repeated measures ANOVA for the effect of depth (layer) and group (addition of oil and dispersant) on the response of variables.

Variables	Depth			Group			Depth × group			Error	
	df	MS	*F*	df	MS	*F*	df	MS	*F*	df	MS
TB	2	0.217	1.251	2	2.552	14.682***	4	0.365	2.099	234	0.174
HF	2	81.478	5.333**	2	32.962	4.157*	4	6.974	0.456	234	15.279
PHYTO	2	56.637	12.231***	2	100.47	21.698***	4	15.701	3.391**	234	4.630
Chl *a*	2	1.107	7.535**	2	2.045	13.913***	4	0.760	5.172**	234	0.147
ZOO	2	123.78	8.030***	2	65.189	4.299*	4	9.091	0.590	234	15.416
WT	2	1.086	2.491	2	0.750	1.721	4	7.35×10^3	0.017	243	0.436
SAL	2	3.39×10^3	0.293	2	0.121	10.443***	4	5.33×10^3	0.461	243	1.16×10^2
pH	2	8.36×10^4	0.400	2	1.78×10^2	8.940***	4	4.55×10^5	0.022	243	2.09×10^3
DO	2	2.01×10^2	0.046	2	3.248	7.491***	4	4.06×10^2	0.094	243	0.434
DIN	2	3.84×10^2	0.144	2	0.453	1.702	4	0.111^2	0.417	243	0.266
DIP	2	0.331	0.997	2	0.305	0.918	4	0.322	0.970	243	0.332
DSi	2	0.371	0.433	2	1.368	1.599	4	0.714	0.835	243	0.855
DOC	2	0.271	1.228	2	8.110	36.738***	4	0.377	1.708	243	0.221
TPH	2	1.29×10^7	6.576**	2	1.57×10^8	80.023***	4	1.23×10^7	6.277***	243	1.96×10^6

TB: total bacteria; HF: heterotrophic flagellates; Chl *a*: chlorophyll *a*; PHYTO: phytoplankton; ZOO: zooplankton; WT: water temperature; SAL: salinity; DO: dissolved oxygen; DIN: dissolved inorganic nitrogen; DIP: dissolved inorganic phosphorus; DSi: dissolved silica; DOC: dissolved organic carbon; TPH: total petroleum hydrocarbon; df: degrees of freedom for the factors; MS: values of mean square.

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

incubated for 24 h in the dark at 4 °C. Finally, the chlorophyll *a* concentration was estimated in accordance with the equation of Humphrey and Jeffrey [12]. To analyse dissolved organic carbon (DOC), 10-mL water samples were filtered through a GF/F filter and analysed using high-temperature catalytic combustion (TOC-V_{CPH}, Shimadzu, Japan).

The total petroleum hydrocarbon (TPH) concentration was determined in accordance with Kim et al. [10]. Seawater was collected from a mesocosm at the three depths mentioned above and transferred into a Teflon-lined glass bottle that had been precleaned with dichloromethane and hexane after baking at 400 °C for 4 h. To analyse residual oil content in the samples using a portable fluorometer, 20–1000 mL of seawater was extracted with hexane. The hexane extract was transferred into a 13 × 100 mm quartz cuvette and the fluorescence of the extract was measured using a portable fluorometer (10AU, Turner Designs, USA) equipped with a low-pressure mercury vapour lamp and a standard 300–650 nm photomultiplier tube. Standard solutions for calibration were prepared with Iranian heavy crude oil, which was identified as the major component of the oil spilled from oil tanker *M/V Hebei Spirit* accident after GC fingerprinting by the Oil and POPs Research Group, KORDI [13].

2.3. Analysis of planktonic communities

To analyse planktonic components, subsamples were taken daily from each mesocosm at three depths at 9:00 AM using the peristaltic pump. To analyse total bacteria and heterotrophic flagellates (HF), 30-mL samples were collected in 50-mL sterilized PE bottles and preserved immediately with buffered glutaraldehyde at a final concentration of 2%. The samples were stored in the dark at 4 °C until further processing. The fixed cells of total bacteria were filtered onto black polycarbonate filters (GTBP 02500, Millipore, Ireland) and stained with DAPI (4′,6-diamidino-2-phenylinodole) solution at a concentration of 1 μg/mL [14]. For the stained bacteria, at least 600 cells were counted per sample by epifluorescence microscopy (Axioplan microscope, Zeiss, Germany) at a magnification of ×1000. For HF, at least 200 cells were counted by epifluorescence microscopy at a magnification of ×400–1000 following ultraviolet excitation by the primuline staining method [15]. To analyse phytoplankton, 500-mL samples were collected, fixed immediately with Lugol's solution at a final concentration of 5%, and concentrated for 24 h by sedimentation. Cell counts and phytoplankton identification were performed for at least 500 cells per sample using a Sedgwick–Rafter counting chamber under a light microscope (Axioplan, Zeiss) at ×400 magnification. Ciliates were not analysed in the present study because there were few present throughout the experiment, even though ciliates are one of the most important trophic components that bridge the gaps between primary producer, decomposer, and consumer. To determine the viability of zooplankton, 2-L samples were collected in 3-L acid-cleaned PE bottles and examined immediately using a dissecting microscope (Discovery V8; Zeiss, Germany). Zooplankton was considered to be dead and was not counted when they showed no movement after being touched with a needle.

2.4. Statistical analysis

Results are presented as the mean ± standard deviation (SD). Experimental groups and/or layers were compared by two-way or one-way analysis of variance (ANOVA), followed by Scheffe's post hoc test. *p* Values less than 0.05 were considered significant. To examine the relationships between measured parameters, Pearson's correlation analysis was applied. Cross-correlation analysis enabled us to discover changes in biotic/abiotic factors over time after introduction of pollutant. These data were then transformed into normalized metadata, whereby the time-series data ranged between −1 and 1. SPSS for Windows (ver. 13) and XLSTAT 2011 programs were used to analyse this dataset.

3. Results

3.1. Changes in TPH

Variations in TPH concentration were significantly different among both experimental groups and depths (Table 1). The concentration of TPH was higher in the O + D group than in the OIL group (Figs. 2 and 3). In particular, after the addition of oil plus dispersant TPH was dispersed rapidly to the bottom layer of the water column: in the surface layer, the mean TPH concentration was 3687 ± 3350 μg/L, whereas the TPH concentrations in the middle and bottom layers were 2088 ± 1309 μg/L and 1349 ± 934 μg/L, respectively (Fig. 4). However, in the OIL group TPH was dispersed slowly into the bottom layer; it reached a concentration of 24.74 ± 11.50 μg/L there, compared with a concentration of

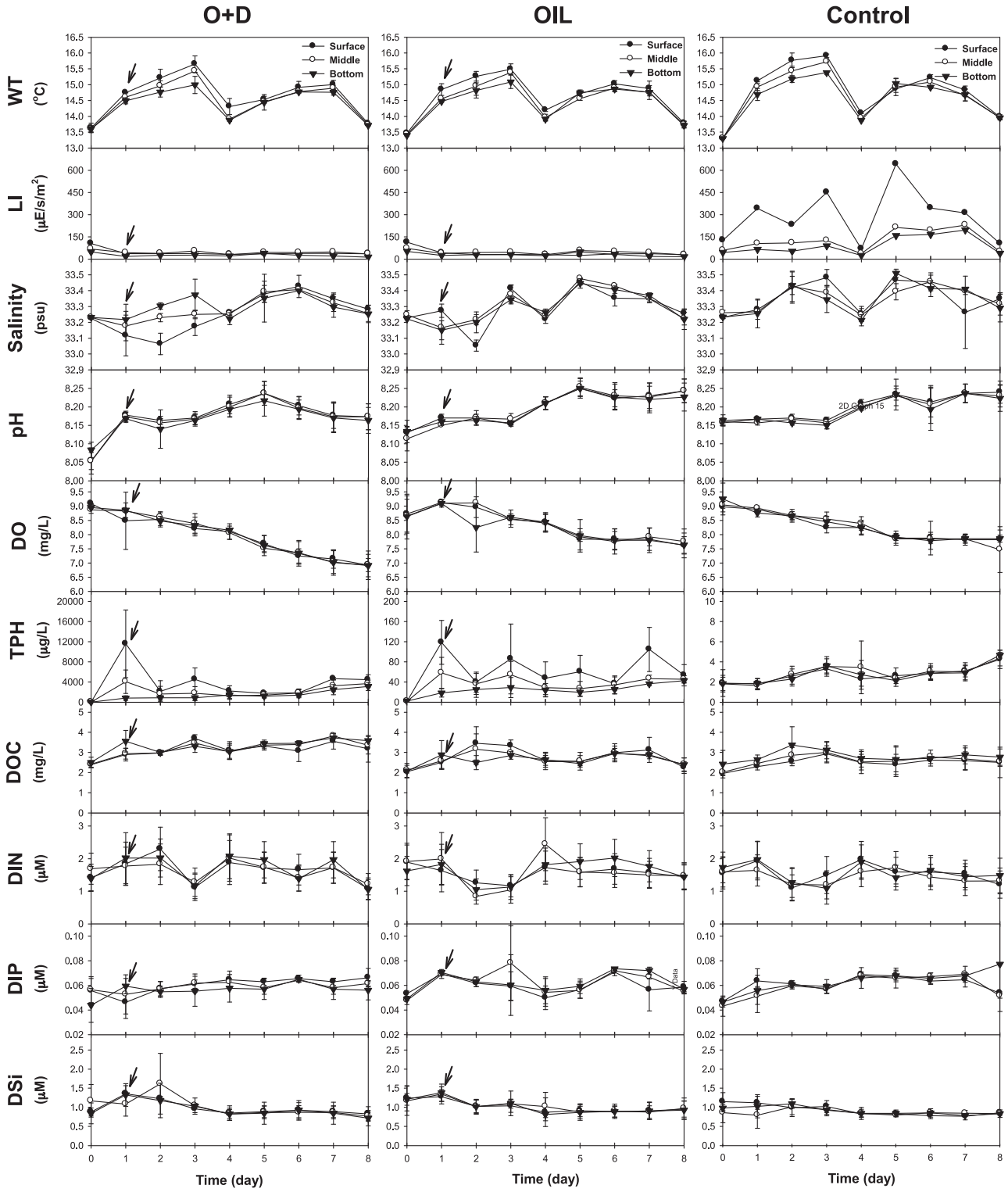

Fig. 2. Changes in abiotic factors among groups and depths in mesocosms. O + D: group subjected to the addition of crude oil plus dispersant; OIL: group subjected to the addition of crude oil only. WT: water temperature; DO: dissolved oxygen; DIN: dissolved inorganic nitrogen; DIP: dissolved inorganic phosphorus; DSi: dissolved silica; DOC: dissolved organic carbon; TPH: total petroleum hydrocarbon. Data represent mean ± SD from three independent assays. Arrows indicate the day on which oil was added.

61.00 ± 36.50 μg/L at the surface. In the control group, negligible levels were found in all layers.

3.2. Changes in environmental factors

The daily light intensity during the experimental period is shown in Fig. 2. The penetration of light was reduced severely by the film of oil. Light intensities were not significantly different between the O + D and OIL groups, with mean values of 39 ± 20 μE/s/m^2 and 38 ± 18 μE/s/m^2 ($p > 0.05$, one-way ANOVA), respectively. However, the mean intensity in the control group was 170 ± 145 μE/s/m^2. Changes in water temperature were not significantly different among groups and depths (Table 1). Salinity, pH, and DO concentrations differed significantly among experimental

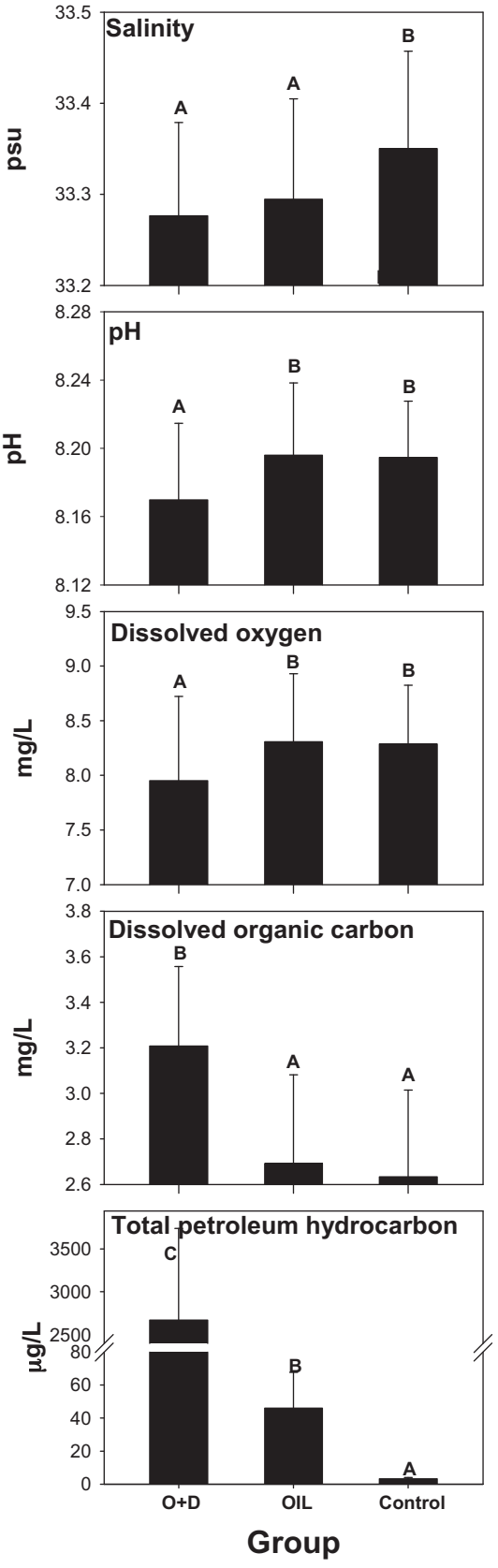

Fig. 3. Changes in the mean levels of abiotic factors that were determined to show significant differences among groups. O + D: group subjected to the addition of crude oil plus dispersant; OIL: group subjected to the addition of crude oil only. Data represent mean ± SD from three independent assays. Results were analysed by one-way ANOVA and Scheffe's post hoc tests. Letters (A, B, and C) indicate significant differences among experimental groups ($p < 0.05$).

groups ($p < 0.05$), but not among layers within a group (Table 1, Fig. 3). Salinity was found to be lower in the O + D and OIL groups than in the control group. pH decreased rapidly after the addition of oil plus dispersant, whereas its value decreased slowly in the OIL and control groups during the experimental period. Variation in DO concentration showed a similar trend to that of pH. In the O + D group, the concentration of DOC increased from 2.40 mg/L to 3.18 mg/L over the nine days, whereas the concentrations in the OIL and control groups increased less, from 2.08 mg/L to 2.35 mg/L, and 2.13 mg/L to 2.60 mg/L, respectively (Fig. 2). In terms of comparison among layers, in the O + D and OIL groups the DOC concentration was the same throughout water column, but the concentration in the control group was increased significantly to the bottom layer of the water column (Fig. 4). Changes in nutrient levels, in terms of DIN, DIP, and DSi, were not significantly different among groups and depths during the entire experimental period (Table 1 and Fig. 2).

3.3. Changes in phytoplankton communities due to oil spill dispersants

Before the addition of crude oil, the phytoplankton communities in all the mesocosms were similar to that in ambient seawater (data not shown). The mean abundance of phytoplankton was determined, namely, a density of $7.40 \pm 2.45 \times 10^4$ cells/L, and 36 species were identified and divided into two taxonomic groups: diatoms of 29 species and dinoflagellates of 7 species (Fig. 5). A centric diatom, *Chaetoceros diadema*, was the predominant phytoplankton with a mean abundance of $2.37 \pm 1.03 \times 10^4$ cells/L (mean proportion of the total: 32.07%). Besides *C. diadema*, the diatoms *Skeletonema* cf. *marioni* (17.03%), *Guinardia delicatula* (14.90%), and *Leptocylindrus danicus* (9.45%) each comprised a substantial proportion over 5% of the total phytoplankton abundance (Fig. 6). After the introduction of crude oil, the dynamics of the phytoplankton community was significantly different among groups as well as among layers of the water column ($p < 0.05$, Table 1). The abundance of phytoplankton in the O + D group decreased rapidly and reached $1.09 \pm 0.24 \times 10^4$ cells/L on day 8 (Figs. 5 and 7), and the predominant species changed from *C. diadema* (7.99%) to *G. delicatula* (26.47%). Furthermore, *Thalassionema nitzschioides*, a pennate diatom, showed a particularly marked change to 22.28% of the total (Fig. 6). In the OIL and control groups, the mean abundances on day 8 decreased slightly to densities of 5.13 ± 0.71 and $5.61 \pm 1.58 \times 10^4$ cells/L, respectively (Figs. 5 and 7). In addition, *C. diadema* remained the predominant species continuously at the water column and showed a marked increase in proportion to over 80% in both groups (Fig. 6).

With regard to the changes at different depths in the water column, in the O + D group, the abundance of phytoplankton decreased rapidly at the surface ($2.09 \pm 0.97 \times 10^4$ cells/L) and in the middle layer ($2.56 \pm 2.04 \times 10^4$ cells/L), whereas in the bottom layer, it decreased less to a density of $5.08 \pm 2.53 \times 10^4$ cells/L (Fig. 8). In the OIL group, the abundance of phytoplankton decreased at the surface and middle, but it was hardly affected in the bottom layers (Fig. 8). In the control group, phytoplankton abundance did not differ significantly among the layers ($p > 0.05$, Fig. 8). The composition of phytoplankton in the different layers was changed in a manner dependent on TPH concentration. After the addition of oil plus dispersant, the predominant species in the water column changed from *C. diadema* to *G. delicatula* and *T. nitzschioides*. In contrast, *C. diadema* remained the predominant species in all layers in the OIL and control groups (Fig. 6).

Chlorophyll *a* concentration and phytoplankton abundance showed a positive correlation ($r = 0.48$, $p < 0.001$). Chlorophyll *a* concentration differed significantly among groups and depths during the experimental period (Table 1): chlorophyll *a* concentration was lower in the O + D group, with a mean concentration of

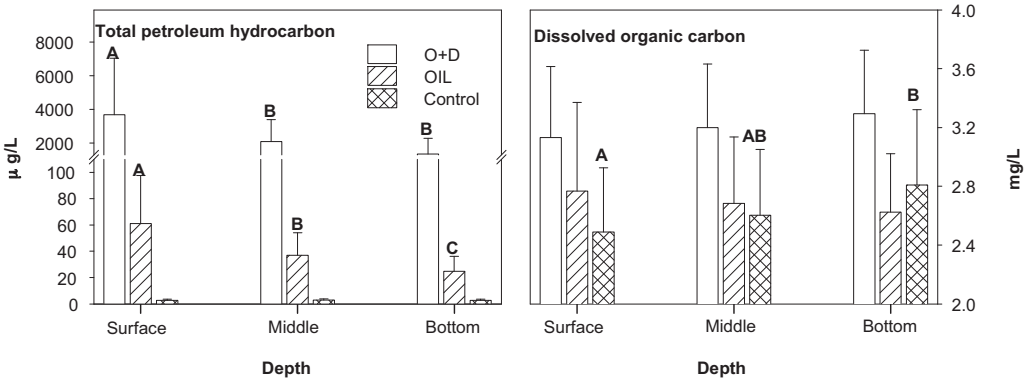

Fig. 4. Changes in the mean levels of total petroleum hydrocarbon and dissolved organic carbon that showed significant differences among depths over the entire experimental period. O + D: group subjected to the addition of crude oil and dispersant at concentrations of 1000 and 100 ppm (v/v), respectively; OIL: group subjected to the addition of crude oil at a concentration of 1000 ppm (v/v). Data represent mean ± SD from three independent assays. Results were analysed by one-way ANOVA and Scheffe's post hoc tests. Letters (A, B and C) indicate significant differences among layers ($p < 0.05$).

0.5 ± 0.4 μg/L, than in the OIL and control groups (Fig. 7). With regard to the different layers in the water column, in the O + D group, chlorophyll *a* was completely absent at the surface on day 6 and in the middle layer on day 7. However, the opposite changes were observed in the OIL and control groups, namely, increases over time (Fig. 5).

3.4. Dynamics of bacteria, flagellate, and zooplankton communities after exposure to chemical dispersants

The density of total bacteria after exposure to crude oil plus dispersant increased rapidly for the first two days and then decreased for the rest of the experimental period (Fig. 5). The densities of bacterial cells increased slightly in OIL and control groups, with no significant difference between them (Fig. 7). With regard to differences among the layers of the water column, bacterial cells showed the opposite trend to that of phytoplankton abundance (Fig. 8): bacterial cells increased rapidly at the surface in the O + D group, whereas the density in the middle and bottom layers increased slightly. The density of HF was affected slightly by exposure to oil or oil plus dispersant (Fig. 5 and 7), but they showed changes deeper in the water column (Fig. 8). The time lag the changes in HF might be associated with fluctuation in the total density of all bacteria: the total density of bacteria increased, and then the density

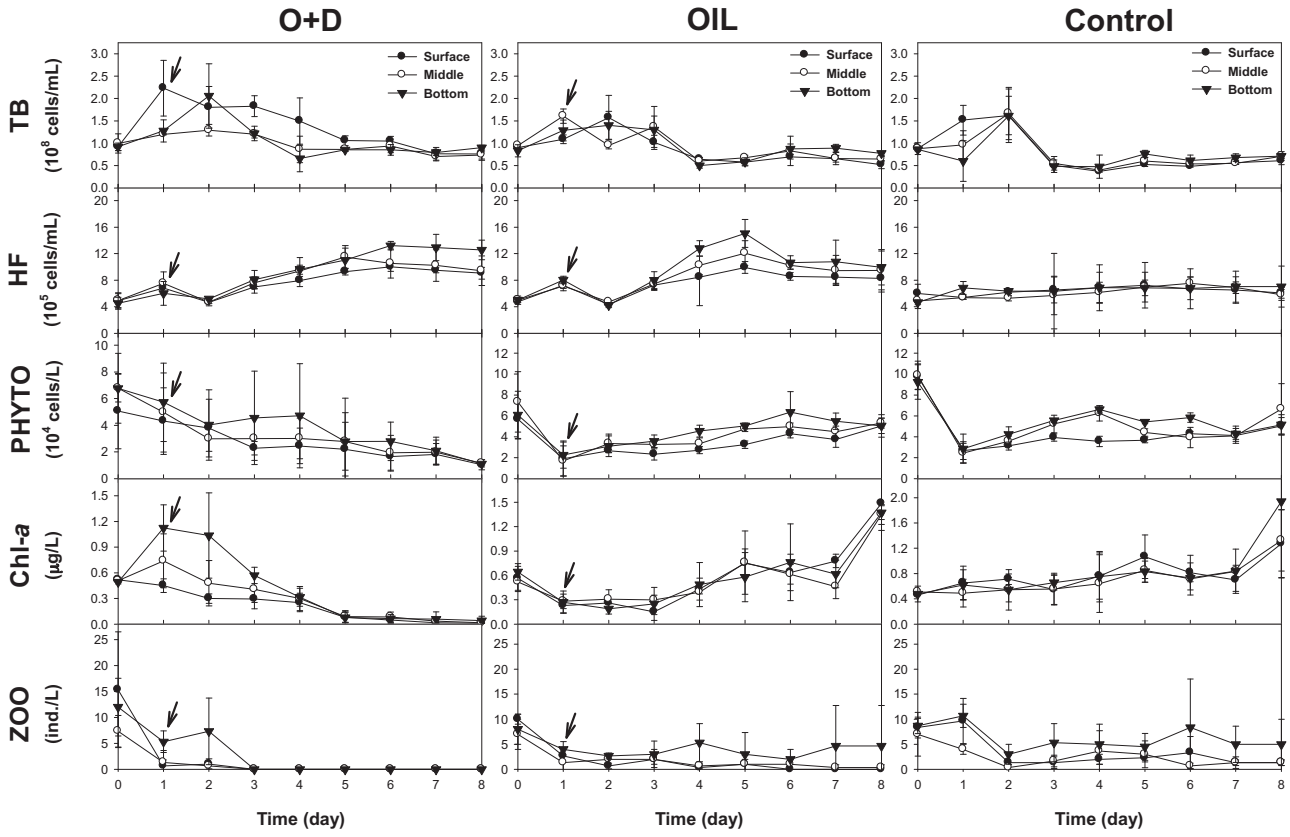

Fig. 5. Changes in biotic factors among groups and depths in mesocosms. O + D: group subjected to the addition of crude oil plus dispersant; OIL: group subjected to the addition of crude oil only. TB: total bacteria; HF: heterotrophic flagellates; PHYTO: phytoplankton; Chl *a*: chlorophyll *a*; ZOO: zooplankton. Data represent mean ± SD from three independent assays. Arrows indicate the day on which oil was added.

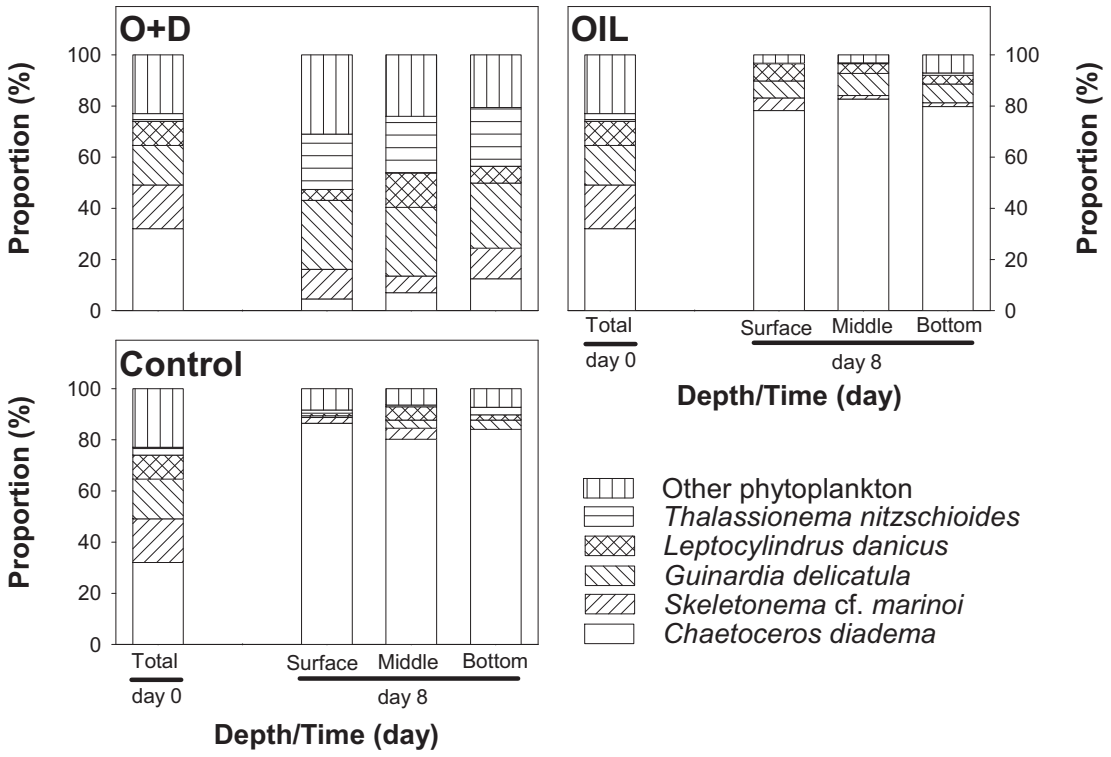

Fig. 6. Mean proportion of phytoplankton divided into taxonomic species. O + D: group subjected to the addition of crude oil and dispersant at concentrations of 1000 and 100 ppm (v/v), respectively; OIL: group subjected to the addition of crude oil at a concentration of 1000 ppm (v/v). Data represent means from three independent assays.

of HF increased gradually thereafter. The zooplankton community was affected severely in the O + D group; it was wiped out completely at all depths two days after exposure (Fig. 5). However, in the OIL group, zooplankton survived longer and living individuals remained on the final day (Fig. 7). In addition, the abundance of zooplankton increased with depth in the OIL group (Fig. 8). In the control group, the abundance of zooplankton changed little during the experimental period.

3.5. Time-delayed impact of TPH

When cross-correlation analysis was applied, a significant relationship between TPH concentration and biotic/abiotic factors that showed a time lag could be identified (Table 2). In the O + D group, the combination between TPH concentration and total bacteria/DOC/DIN/salinity showed positive cross-correlation factors: the combinations of variables analysed showed an interaction with a time lag of two days. In contrast, the variables of TPH and phyto- and zooplankton communities showed a negative cross-correlation with a time lag of one day. In the OIL group, the interaction between TPH and most biotic/abiotic factors occurred slowly with a time lag of five to six days, but zooplankton communities decreased rapidly in association with TPH with a time lag of two days. In the control group, there was no association between TPH and living organisms or environmental factors. The relationship between biotic and abiotic factors is important. With regard to the cross-correlation between biotic and abiotic factors, DOC and DIN had a delayed effect on total bacteria in the O + D group with time lags of five and seven days, respectively, whereas their interaction in the OIL group was rapid with a time lag of one to three days. Nutrient levels in the O + D group hardly affected the proliferation of phytoplankton, but these factors showed a cross-correlation in the OIL and control groups, in which similar trends of positive correlations were shown with time lags of three to seven days.

4. Discussion

4.1. Impact of dispersant on marine environments

Oil that is spilled at sea forms a surface slick, which is dispersed naturally into the water to some extent by wave action and ocean turbulence. Natural dispersion of heavy crude oil is relatively slow. However, when a chemical dispersant is added to an oil spill, it is dispersed rapidly into the bottom layer of the water column. McAuliffe et al. [16] pointed out that the concentration of TPH in spilled heavy crude oil alone was lower, with a maximum of around 1 ppm, at a depth below one meter; However, given that more oil is taken down into the water column as opposed to staying as a slick on the surface when dispersants are used, concentrations of TPH will often be higher at greater depths in the water column, at concentrations of approximately 20–100 ppm, than they would be without dispersants. In the present study, in the O + D group, the concentrations of TPH in the subsurface waters near the bottom were higher than those in the OIL and control groups. Measurements of temperature confirmed that the mesocosms studied were filled with nearly identical masses as compared with ambient water. pH, DO and salinity were identical masses in a mesocosm, but their concentrations were significantly different among groups. Slower changes in pH of the O + D group as compared with other groups are a direct result of inhibition of phytoplankton growth [17]. Under these experimental conditions, pH changes in the enclosed seawater can be in agreement with phytoplankton growth. Dissolved oxygen more decreased in the O + D group than that of OIL group. This variation might be explained by bacterial activity [11]. However, these minor changes are assumed to have had little effect on the microbial community responses in the mesocosms. The decrease in observed salinity in the O + D and OIL groups as compared with the control group can be explained by the inhibition of water evaporation by an oil slick: water cannot evaporate easily through a layer of oil of approximately 5 mm in

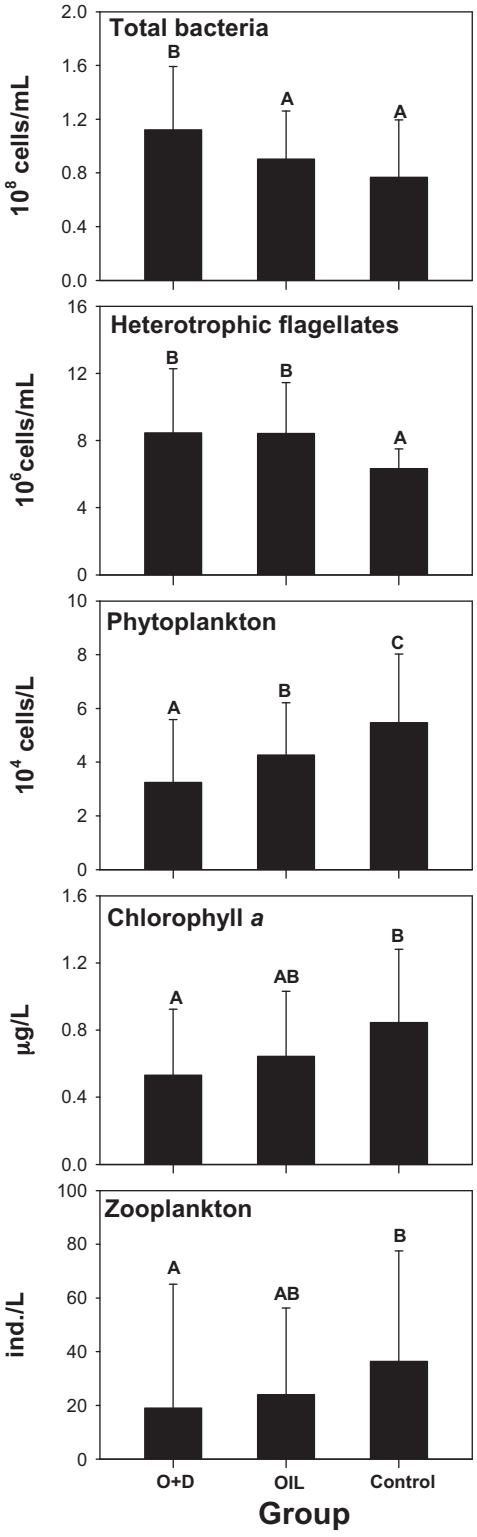

Fig. 7. Changes in the mean levels of biotic factors that were determined to show significant differences among groups. O + D: group subjected to the addition of crude oil plus dispersant; OIL: group subjected to the addition of crude oil only. Data represent mean ± SD from three independent assays. Results were analysed by one-way ANOVA and Scheffe's post hoc tests. Letters (A, B, and C) indicate significant differences among experimental groups ($p < 0.05$).

thickness on the surface. This indicates that water did not easily penetrate thick oil films on the surface. Moreover, direct light penetration through the oil film on the water surface was negligible over the up side. In particular, the concentration of DOC was increased in the O + D group as compared with the OIL and control groups. This resulted in an increase in DOC when active biodegradation was proceeded [18]. Nutrient concentrations are important to dynamics of microbial organisms. Measurements of nutrient levels confirmed that all mesocosms in the study were filled with nearly identical water masses. Colwell and Walker [19] reported that the levels of nitrogen and phosphorus in seawater might limit microbial degradation of oil. However, in the present study, DIP concentrations increased over time. This might be explained by the following factors: (1) decomposition of organic particles from dead phytoplankton and zooplankton, and (2) a decrease in the consumption of DIP by bacteria and phytoplankton [11,20].

4.2. Impact of dispersant on marine planktonic communities

In the present study, the most evident effect in the O + D and OIL groups was the rapid stimulation of bacterial growth, which was similar to the findings of the study of MacNaughton et al. of an oil spill microcosm [21]. This rapid effect was probably due to the immediate availability of low-molecular-weight fractions of effluent that could act as sources of carbon and energy without the requirement for any period of bacterial adaptation [22]. Atlas and Bartha [20] observed that the biodegradation of petroleum can increase the level of available energy sources with a resulting increase in the abundance of bacteria. In the O + D and OIL mesocosms that were analysed in the present study, changes in bacterial abundance involved an initial increase, followed by a decrease for the rest of the experimental period in spite of the supplementation of effluent nutrients. Given that the initial increase in bacterial abundance was followed by an increase in the abundance of HF, the latter might graze on bacteria as a food source. These flagellates constitute a substantial fraction of microplankton biomass in the sea [23]. In addition, HF became more abundant upon the addition of oil or oil plus dispersant, which was probably due to the increased availability of bacteria as a food source. Thus, bacteria and HF might show tolerance to dispersant or TPH released from crude oil. As mentioned above, bacteria could grow rapidly during the early period owing to an abundance of energy sources. Subsequent progress in the microbial loop might result in a further increase in predators that feed on bacteria.

In the present study, phytoplankton abundance was decreased severely upon the use of chemical dispersant. Moreover, this abundance differed according to depth. Our results indicate that the addition of chemical dispersant to remove heavy crude oil can deplete phytoplankton communities severely owing to the rapid dispersion of TPH in comparison with the effects of crude oil alone. The effects of the dispersant include immediate toxicity from the soluble aromatic fraction and altered physico-chemical conditions below the floating oil (for example, rapid penetration into the water column as a result of the dispersant) [2]. Nutrient levels are also one of the most important factors that control the growth of phytoplankton. Miller et al. [24] stated that phytoplankton communities cannot grow in areas contaminated with oil because nutrient levels are already low due to the monopolization of nutrients by bacteria. However, in the present study, nutrients were not exhausted in the O + D and OIL groups because the growth of bacteria, which were the major consumer of nutrients, was limited by feeding pressure from HF. In contrast to the result of Miller et al. [24], phytoplankton communities were probably not affected by nutrient levels under the conditions of the present study.

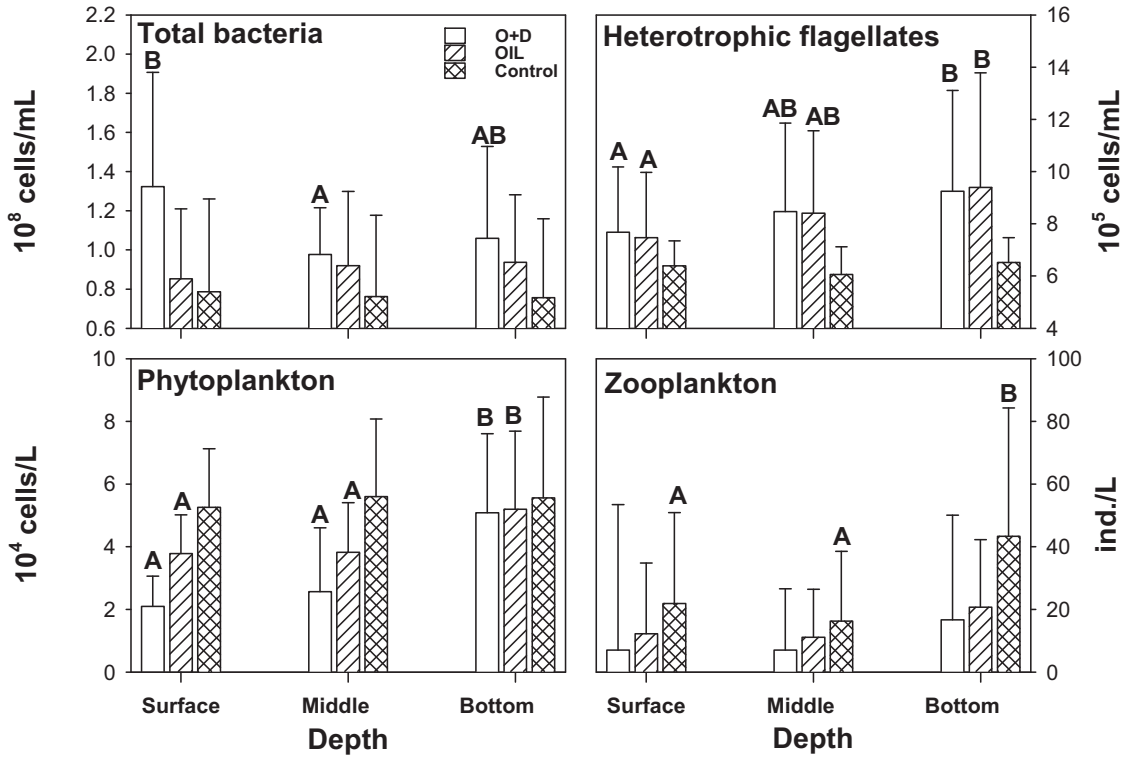

Fig. 8. Changes in the mean levels of biotic factors that showed significant differences among layers over the entire experimental period. O + D: group subjected to the addition of crude oil and dispersant at concentrations of 1000 and 100 ppm (v/v), respectively; OIL: group subjected to the addition of crude oil at a concentration of 1000 ppm (v/v). Data represent mean ± SD from three independent assays. Results were analysed by one-way ANOVA and Scheffe's post hoc tests. Letters (A, B and C) indicate significant differences among layers ($p < 0.05$).

In terms of the succession of phytoplankton, the predominant species changed from *C. diadema* (centric diatom) to *G. delicatula* (centric diatom) and *T. nitzschioides* (pennate diatom) after the introduction of oil plus dispersant. Phytoplankton from different systematic taxa might show different responses and tolerances to oil. In general, exposure to oil inhibits phytoplankton growth, but some signs of its stimulation of growth have also been documented [25]. Evidence of differences in the effects of oil on phytoplankton groups has also been reported by Davenport [26]. Thus, tolerance to the effects of oil might vary among phytoplankton species. Variation in tolerance among phytoplankton species will result in the succession of phytoplankton communities and produce different ecosystems.

Zooplankton communities responded rapidly to the addition of crude oil plus dispersant, as compared with oil alone or the control: in the O + D group, zooplankton communities collapsed, whereas in the OIL and control groups, they showed slight decreases. Barron and Káaihue [27] reported that most zooplankton die upon contact with dispersed oil. The major routes of contamination of zooplankton are direct uptake from the water which leads to changes in the metabolic rates of zooplankton, uptake from oil contaminated food (important for copepods in particular), or ingestion of oil particles, which can resemble food items in terms of size [28]. Another reason for the disruption of zooplankton communities is that, in spills, zooplankton suffers direct mortality as a result of the contamination of tissue by aromatic compounds [29]. This indicates that, in open water, the wide distribution of zooplankton and rapid change of water masses promote the recovery of zooplankton communities after oil spills, whereas in enclosed water bodies, such as estuaries and bays, recovery might take notably longer [28].

5. Implications for the responses of marine ecosystems upon treatment with dispersant

The environmental acceptability of dispersants remains an important question. Although there have been many advances in dispersant formulations and methods of application over the last 30 years, formation of dispersed droplets by use of dispersants still remain some obstacles of perception [6,30]. Fig. 9 made by cross-correlation analysis shows the variations in planktonic communities and environments that were observed in the present study after mesocosms were exposed to an oil spill. Similar changes might occur in a marine ecosystem if oil was spilled in enclosed coastal waters. Although chemical dispersant can remove spilled crude oil, the exact composition and state of the crude oil that has been spilled must be considered. For example, a heavy crude oil will almost never disperse naturally into the water column because of its low volatility and high viscosity, whereas light crude oil will disperse naturally in turbulent seas [31]. If a large amount of heavy crude oil is spilled in the sea, physical treatments (i.e., use of adsorbent substances) to remove the oil might be better than the use of a chemical dispersant, even though it might take longer for the oil to be removed. These removals of the crude oil have important advantages, because the physical treatments might impose less of an environmental impact on the ecosystem. The results in Fig. 9 show that the use of a chemical dispersant could destroy marine planktonic ecosystems rapidly, as well as cause environments to deteriorate. After dispersants were used in an area with an oil spill, the most important producers in the marine ecosystem, communities of phytoplankton and zooplankton, were killed off almost completely within one day, but upon the introduction of crude oil alone, marine organisms survived longer and environments

Table 2
Summary of significant cross-correlation between total petroleum hydrocarbon and biotic/abotic factors. Significant cross-correlation coefficients (CCF) are given as r and its sign.

Group	Input variable	Output variable	Lag time (day)	Sign	CCF	r for $p < 0.05$
O + D	TPH	DOC	1	+	0.246	0.195
		DIN	1	+	0.272	0.195
		DO	1	−	−0.327	0.195
		SAL	2	+	0.284	0.195
		TB	2	+	0.382	0.195
		HF	6	+	0.426	0.195
		PHYTO	1	−	−0.212	0.195
		ZOO	1	−	−0.196	0.195
	DOC	TB	7	+	0.249	0.195
	DIN	TB	5	−	−0.241	0.195
	DIP	TB	1	+	0.349	0.195
	TB	DO	3	−	−0.467	0.195
		HF	1	+	0.347	0.195
	PHYTO	DOC	4	+	0.220	0.195
		DIN	8	+	0.293	0.195
		DIP	6	+	0.234	0.195
		DSi	8	+	0.242	0.195
		TB	5	+	0.320	0.195
OIL	TPH	DOC	5	+	0.197	0.195
		DO	6	−	−0.330	0.195
		SAL	2	+	0.297	0.195
		TB	3	+	0.198	0.195
		HF	5	−	−0.388	0.195
		PHYTO	6	−	−0.254	0.195
		ZOO	2	−	−0.208	0.195
	DOC	TB	1	+	0.281	0.195
	DIN	TB	3	+	0.340	0.195
		PHYTO	3	+	0.320	0.195
	DIP	TB	4	+	0.283	0.195
		PHYTO	3	+	0.275	0.195
	DSi	PHYTO	7	+	0.267	0.195
	TB	DO	3	+	−0.427	0.195
		HF	2	+	0.359	0.195
	PHYTO	DOC	3	+	0.201	0.195
		TB	5	+	0.448	0.195
Control	DOC	TB	1	+	0.304	0.195
		PHYTO	5	+	0.389	0.195
	DIN	TB	2	+	0.252	0.195
		PHYTO	1	+	0.376	0.195
	DIP	TB	5	+	0.254	0.195
		PHYTO	4	+	0.196	0.195
	DSi	PHYTO	8	+	0.209	0.195
	TB	DO	2	−	−0.367	0.195
		HF	6	+	0.227	0.195
	PHYTO	DOC	5	+	0.389	0.195
		TB	6	+	0.693	0.195
		ZOO	7	+	0.429	0.195

TB: total bacteria; HF: heterotrophic flagellates; PHYTO: phytoplankton; ZOO: zooplankton; WT: water temperature; SAL: salinity; DO: dissolved oxygen; DIN: dissolved inorganic nitrogen; DIP: dissolved inorganic phosphorus; DSi: dissolved silica; DOC: dissolved organic carbon; TPH: total petroleum hydrocarbon. O + D: group subjected to the addition of crude oil and dispersant at concentrations of 1000 and 100 ppm (v/v), respectively; OIL: group subjected to the addition of crude oil at a concentration of 1000 ppm (v/v).

changed less than when dispersant was also added. However, the use of dispersant has some advantages in specific contaminated areas. For example, the removal of crude oil by the addition of dispersants reduces the environmental impact of oil that could contaminate shorelines. Dispersants reduce the environmental impact of spilled oil, thereby preventing oil from impacting shorelines and sensitive habitats [7]. In addition, the study of Gilfillan et al. [32] showed that the negative effects of untreated oil coming ashore will be worse in general than any effects of the dispersion of that same oil: the results showed clearly that there was no residual oil in sediment exposed to dispersed oil and no resulting mortality to organisms following one tidal cycle [7]. In every case in which an oil spill has had a significant impact, it has been caused by oil coming into a near-shore or inter-tidal zone [30].

To use the various tools that can be implemented in response to an oil spill in an optimal manner, responders must conduct extensive preplanning, must be prepared to base decisions on the concept of net environmental benefit, and must remain open-minded (i.e., not rule out certain response options in advance) [7]. In this manner, the common objective of mitigating the spill

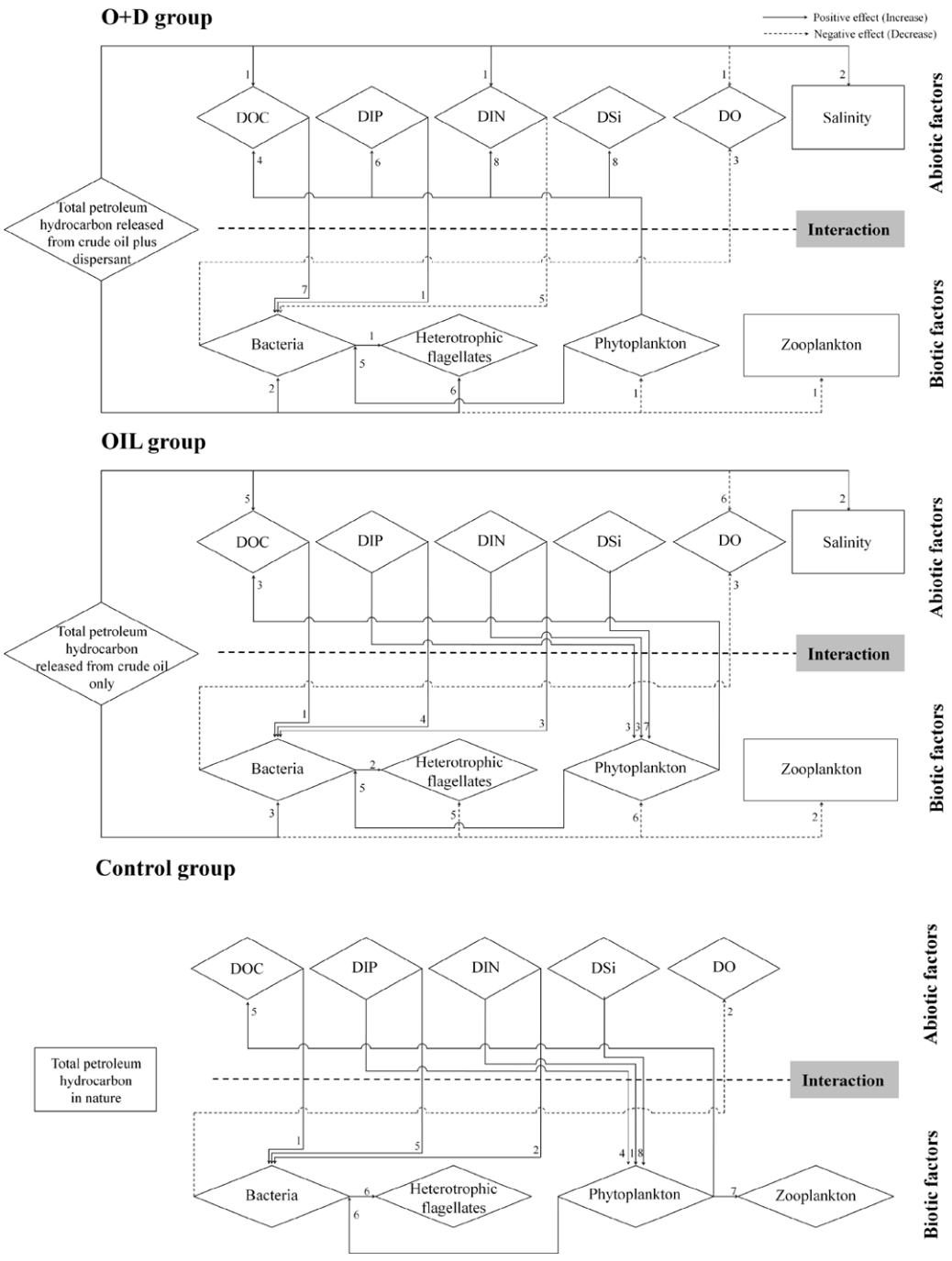

Fig. 9. Schematic diagram that summarizes the variations in biotic and abiotic factors in an oil spill by cross-correlation analysis. The solid and dotted lines indicate positive (increase) and negative (decrease) correlation, respectively. DO: dissolved oxygen; DIN: dissolved inorganic nitrogen; DIP: dissolved inorganic phosphorus; DSi: dissolved silica; DOC: dissolved organic carbon. The numerals on the arrows are time lags (day).

while minimizing the overall impact on sensitive resources can be accomplished.

It is essential to evaluate how crude oil used with chemical dispersant can be dispersed and dissolved in nature. To evaluate this, pathway of exposure of crude oil should be monitored, and their ecological risk assessment test also should be carried out in artificially expended ecosystem (macrocosm) and nature.

Acknowledgements

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